

Dovepress

JOURNAL OF BLOOD MEDICINE

VOLUME 14 - 2023 - OPEN ACCESS



Taylor & Francis
Taylor & Francis Group

Table of Content

Cerebral Venous Sinus Thrombosis induced By Hypercoagulation in Patient With Systemic Lupus Erythematosus: A Case Report and Literature Review

Lisda Amalia

Pages: 1-6

Role of Hemin in the Immune Response of T Follicular Helper Lymphocytes Expressing T-Cell Immunoreceptor with Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Domains, Programmed Cell Death-1, and Interleukin-21 in Allo-Auto Positive and Negative Thalassemia

Betty Agustina Tambunan, I Dewa Gede Ugrasena & Aryati

Pages: 7-17

Prevalence of ABO and Rh Blood Group Among Volunteer Blood Donors at the Blood and Tissue Bank Service in Addis Ababa, Ethiopia

Getu Jenbere Debele, Fekadu Urgessa Fita & Melatwork Tibebe

Pages: 19-24

Platelet Parameters and Their Correlation with Parasitemia Levels Among Malaria Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia: Comparative Cross-Sectional Study

Haftu Asmerom, Kabtamu Gemechu, Tilahun Bete, Beza Sileshi, Berhe Gebremichael, Muluken walle & Mesay Arkew

Pages: 25-36

Outcomes of Hydroxyurea Accessed via Various Means and Barriers Affecting Its Usage Among Children with Sick Cell Anaemia in North-Western Tanzania

Emmanuela E Ambrose, Benson R Kidenya, Mwesige Charles, Joyce Ndunguru, Agnes Jonathan, Julie Makani, Irene K Minja, Paschal Ruggajo & Emmanuel Balandya

Pages: 37-47

Successful CNS-Centric Therapeutic Management and Genomic Profiling of Primary Cranial Vault Diffuse Large B-Cell Lymphoma

Jordan Davis, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Liuyan Jiang, Vivek Gupta, Ephraim Parent & Han W Tun

Pages: 49-55

Association Between Helicobacter pylori Infection and Anemia Among Adult Dyspeptic Patients Attending Kiryandongo General Hospital, Uganda

Daisy Asimwe, Isaac Bangi, Josphe Esanyu, Daniel Ojok, Benedict Okot, Clinton Olong, Robert Wagubi, Godfrey Kisembo, Fred Sempijja, Enoch Muwanguzi & Benson Okongo

Pages: 57-66



Serum Ferritin Levels and Other Associated Parameters with Diabetes Mellitus in Adult Patients Suffering from Beta Thalassemia Major

Ahmed Saleh Ibrahim, Azza Hasan Abd El-Fatah, Ayman Fathy Abd El-Halim & Farid Fawzy Mohamed

Pages: 67-81

Anemia Among Women Using Family Planning at Public Health Facilities in Ambo Town, Central Ethiopia: Multi-Center Cross-Sectional Study

Shalama Lekasa Nagari, Gudina Egata, Ame Mehadi, Tahir Ahmed Hassen, Temam Beshir Raru, Mohammed Abdurke, Mohammed Yuya, Shemsedin Abdulkadir, Hiwot Berhanu & Kedir Teji Roba

Pages: 83-97

MicroRNAs as Quality Assessment Tool in Stored Packed Red Blood Cell in Blood Banks

Zewudu Mulatie, Melak Aynalem & Solomon Getawa

Pages: 99-106

Published online: 08 Feb 2023

Anticoagulation Management in Patients Receiving Warfarin at Private Cardiac Centers in Addis Ababa, Ethiopia

Roza Getachew, Tamrat Assefa Tadesse, Bekele Alemayehu Shashu, Amsalu Degu & Getachew Alemkere

Pages: 107-117

Identification of Laboratory Biomarkers for Early Detection and Clinical Management of Post-Acute Syndrome Among Survivors of the 2013–2016 West Africa Ebola Outbreak in Sierra Leone

Raoul Emeric Guetiya Wadoun, Stephen Sevalie, Maurice Baimba Kargbo, Andrew Clarke, Sherry Bangura, Mariatu Kargbo, Hawa Mariama Sesay, Abdul H Kamara, Jamil Bangura, Alie F Kamara, Sophie Allieu, Hassan Rogers, Maurizio Mattei, Vittorio Colizzi, Carla Montesano & Edwin JJ Momoh

Pages: 119-132

Genotyping Approach to Predict Coa and Cob Antigens in Thai Blood Donor Populations

Oytip Nathalang, Kamonchanok Asisathaporn, Kamphon Intharanut, Wanlapa Chaibangyang, Nipapan Leetrakool, Supattra Mitundee & Sasitorn Bejrachandra

Pages: 133-140

Optimizing Haemophilia Care in Resource-Limited Countries: Current Challenges and Future Prospects

Annick Ndoumba-Mintya, Yacouba L Diallo, Tagny C Tayou & Dora N Mbanya

Pages: 141-146

Outcome of Primary Mediastinal Large B Cell Lymphoma Treated with RCHOP

Khalid Halahleh, Abeer Yaseen, Isra Muradi, Akram Al-Ibraheem, Iyad Sultan & Mohammad Ma'koseh

Pages: 147-157

Effectiveness of COVID-19 Convalescent Plasma (CCP) During the Pandemic Era: A Literature Review

Usha Rani Kandula, Techane Sisay Tuji, Dinkinesh Begna Gudeta, Kassech Leta Bulbula, Anwar Abdulwahed Mohammad, Ketema Diriba Wari & Ahmad Abbas

Pages: 159-187

Von Willebrand Disease Epidemiology, Burden of Illness and Management: A Systematic Review

Ping Du, Aurore Bergamasco, Yola Moride, Françoise Truong Berthoz, Gülden Özen & Spiros Tzivelekis

Pages: 189-208

Prolonged Exposure to Caplacizumab as Rescue Therapy in Refractory Immune Thrombotic Thrombocytopenic Purpura

George M Rodgers, Misa Berndt, Megan Fonteno & Jeffrey A Gilreath

Pages: 209-211

Validation of the International Consensus Group Criteria for Slide Review Following Automated Complete Blood Count at Jimma Medical Center, Ethiopia

Girum Tesfaye Kiya, Aklilu Getachew Mamo, Sintayehu Asaye Biya, Dejene Gebre Gobena, Natal Demeke & Tilahun Yemane Shenkutie

Pages: 213-220

Critical Assessment of Transfusional Practices in the Obstetrics and Gynecology Department At the University Hospital of Kinshasa

Yvon Wangi Ngoy, Mireille Solange Nganga Nkanga, Guy Ndelembo Baina, Audrey Katende Katende, Jacques Bikaula Ngwidiwo, Lethy Mpudi Massamba, Fanny Walo Diessa, Bienvenu Kuyangisa Boloko, Héritier Mawalala Malengele & Benjamin Longo-Mbenza

Pages: 221-231

Pegcetacoplan: A New Opportunity for Complement Inhibition in PNH

Ilene Ceil Weitz

Pages: 239-245

Howell-Jolly Body-Like Inclusions in Coronavirus Disease 2019 (COVID-19): Possible Novel Findings

Amaylia Oehadian, Ian Huang, Andini Kartikasari, Bachti Alisjahbana & Delita Prihatni

Pages: 233-238

Congenital Methemoglobinemia: First Confirmed Case in the Arab Population with a Novel Variant in the CYB5R Gene in the State of Qatar: A Case Report

Abdulrahman Al-Abdulmalek, Reem Al-Sulaiman, Mohammad Abu-Tineh & Mohamed A Yassin

Pages: 247-251

Prevalence of Inherited Thrombophilia in Women with Recurrent Pregnancy Loss During the First Trimester of Pregnancy

Tagwa Yousif Elsayed Yousif

Pages: 253-259

Chronic Myeloid Leukemia, from Pathophysiology to Treatment-Free Remission: A Narrative Literature Review

Ikhwan Rinaldi & Kevin Winston

Pages: 261-277

Managing the Cerebrovascular Complications of Sickle Cell Disease: Current Perspectives

Jennifer Light, Maria Boucher, Jacquelyn Baskin-Miller & Mike Winstead

Pages: 279-293

Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines

Hana Qasim, Alaa Rahhal, Ahmed Husain, Abdelkarim Alammora, Khaled Alsa'ed, Ahmed Abdelghafar Masaad Alsayed, Baha Faiyoumi, Leen Maen AbuAfifeh, Mohammad Abu-Tineh, Awni Alshurafa & Mohamed A Yassin

Pages: 295-302

Military Blood Service in Poland

Tomasz Kryczka, Anna Jedynak, Adam Olszewski, Lidia Sierpinska, Paula Kuzniarska & Dorota Sulejczak

Pages: 309-316

D-Dimer Level Associated with Amount of Sinus involvement Using Digital Subtraction Angiography on Cerebral Venous Thrombosis Patients

Lisda Amalia

Pages: 303-308

RNAi for the Treatment of People with Hemophilia: Current Evidence and Patient Selection

Sara Boyce & Savita Rangarajan

Pages: 317-327

“Severe Anemia: A Case Report of an Uncommon Precipitant of Schizophrenia Relapse”

Krittisak Anuroj, Siwat Chongbanyatcharoen & Romteera Chiencharoenthanakij

Pages: 329-336

Safe Blood Donation from Donors Using Antihypertensive Medication. A Multi-Center Retrospective Quality Study from South-East Norway

Kathrine M Neuman Johnsen, Karin Magnussen, Christian Erstad, Sadaf Nabi Bhatti & Lise Sofie H Nissen-Meyer

Pages: 337-343

A Single Center Study Investigating Clinical Outcomes of Testing for Multiple Myeloma and Immune Deficiency at Low Globulin Levels

Indra Ramasamy

Pages: 345-358

Platelet Selectin Levels in Patients with Cerebral Venous Sinus Thrombosis: Preliminary Findings

Eswi Zenobia, Nushrotul Lailiyya, Sofiati Dian, Cep Juli, Asep Nugraha Hermawan & Lisda Amalia

Pages: 359-365

A Critical Review of Sickle Cell Disease Burden and Challenges in Sub-Saharan Africa

Obi Peter Adigwe, Solomon Oloche Onoja & Godspower Onavbavba

Pages: 367-376

Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines [Letter]

Anumta Ali, Syeda Adeena Zafar & Syeda Sakina Zehra

Pages: 377-378

Switching from Sucrose-Formulated rFVIII to Octocog Alfa (BAY 81-8973) Prophylaxis Improves Bleed Outcomes in the LEOPOLD Clinical Trials

Gili Kenet, Thomas Moulton, Brian M Wicklund, Sanjay P Ahuja, Miguel Escobar & Johnny Mahlangu

Pages: 379-388

Blood Transfusion Complications and Associated Factors Among Blood-Transfused Adult Patients at Debre Markos Comprehensive Specialized Hospital, Ethiopia: A Cross Sectional Study

Yalew Muche, Yemataw Gelaw, Aytenew Atnaf & Zegeye Getaneh

Pages: 389-398

Pharmacokinetic-Pharmacodynamic Comparison of Recombinant and Plasma-Derived von Willebrand Factor in Patients with von Willebrand Disease Type 3

Alexander Bauer, Sofia Friberg-Hietala, Giovanni Smania & Martin Wolfsegger

Pages: 399-411

Pharmacologically Treated Anxiety and Depression in People Diagnosed with von Willebrand Disease: Matched Cohort Study

Katrina Wilcox Hagberg, Susan Jick, Gülden Özen & Ping Du

Pages: 413-425

Efficacy of rFIXFc versus N9-GP Prophylaxis in Patients with Hemophilia B: Matching-Adjusted Indirect Comparison of B-LONG and PARADIGM 2 Trials

Maria Elisa Mancuso, Daniel Eriksson, Aletta Falk, Zalmay Hakimi, Piotr Wojciechowski, Marlena Wdowiak & Robert Klamroth

Pages: 427-434

Systematic Workup of Transfusion Reactions Reveals Passive Co-Reporting of Handling Errors

Elisabeth Nitsche, Jan Dreßler & Reinhard Henschler

Pages: 435-443

Prevalence of Gene Rearrangement by Multiplex PCR in De Novo Acute Myeloid Leukemia in Adult Iraqi Patients

Ali AlJabban & Jaffar Alalsaidissa

Pages: 445-453

Multiply Relapsed Secondary CNS Non-Germinal Center Diffuse Large B-Cell Lymphoma Successfully Treated with CNS-Centric Therapy

Lyndsey L Fournier, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Ke Li, Madiha Iqbal, Vivek Gupta & Han W Tun

Pages: 455-461

Hematological Abnormalities Among Malaria Infected Adult Patients in Association with ABO Blood Groups at Jinella Health Center, Harar, Eastern Ethiopia

Haftu Asmerom, Kabtamu Gemechu, Beza Sileshi & Mesay Arkew

Pages: 463-476

Effect of Moringa oleifera Leaf Powder on Hematological Profile of Male Wistar Rats

Titing Nurhayati, Muhammad Irfan Fathoni, Siti Nur Fatimah, Vita Murniati Tarawan, Hanna Goenawan & Resti Gradia Dwiwina

Pages: 477-485

Factors Influencing Blood Donation Practice Among Health Care Providers of Public Hospitals in Bahir Dar City, North West Ethiopia: A Case Control Study

Belto Tebabal, Tadele Fentabil Anagaw, Ayinengida Adamu & Desta Debalkie Atnafu

Pages: 487-498

Antithrombin Deficiency and Thrombosis: A Wide Clinical Scenario Reported in a Single Institution

Ana Marco-Rico & Pascual Marco-Vera

Pages: 499-506

Autoimmune Hemolytic Anemia Caused by Cold Agglutinin Antibodies in Systemic Lupus erythematosus—a Rare Association: Case Report

Luis Miguel Osorio-Toro, Jhon Herney Quintana-Ospina, Luis Álvaro Melo-Burbano, Paola Andrea Ruiz-Jiménez, Jorge Enrique Daza-Arana, Giovanna Patricia Rivas-Tafurt & Jorge Hernán Izquierdo-Loaiza

Pages: 507-511

Acute Basophilic Leukemia Arising from Chronic Myeloid Leukemia with +8, I(17q)(q10) and der(22)t(9;22) After Imatinib Therapy

Peng Shan, Hang Dong & Shilan Li

Pages: 513-517

Flow Cytometric Enumeration of Peripheral Blood CD34+ Cells Predicts Bone Marrow Pathology in Patients with Less Than 1% Blasts by Manual Count

Tomislav M Jelic, Oscar C Estalilla, Jeffrey A Vos, Gary Harvey, Caitlin J Stricker, Ayodele O Adelanwa, Ahmed A Khalid & Milton J Plata

Pages: 519-535

Correlation Between Hypercoagulable State and Severity Level of Ischemic Stroke With Covid-19 Infection

Lisda Amalia

Pages: 537-542

Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, Borana, Ethiopia

Girma Ashenafi, Melatwork Tibebu, Dagnamyew Tilahun & Aster Tsegaye

Pages: 543-554

The Correlation Between sP-Selectin and Platelet Count in COVID-19 Patients in Referral Hospital, West Java Indonesia

Delita Prihatni, Frany Charisma Budianto, Basti Andriyoko & Suryarini Trisa

Pages: 555-561

Glanzmann Thrombasthenia Associated with Siderotic Synovitis and Arthropathy: A Case Report

Mouhammad J Alawad, Mohammad Abu-Tineh, Awni Alshurafa, Alaa Al-Taie, Anil Yousaf & Mohamed A Yassin

Pages: 563-567

Acquired Clotting Factor Deficits During Treatment with Asparaginase in an Institutional Cohort

Vasiliki Papadopoulou & Giulia Schiavini

Pages: 569-574

Prevalence and Associated Factors of Anemia Among Hospital Admitted Patients in Eastern Ethiopia

Mohammed Umer Yusuf, Nuredin Abdurahman, Haftu Asmerom, Tesfaye Atsbaha, Adisu Alemu & Fitsum Weldegebreal

Pages: 575-588

Deferasirox in Patients with Chronic Kidney Disease: Assessing the Potential Benefits and Challenges

Abdulqadir J Nashwan & Mohamed A Yassin

Pages: 589-594

Patient Blood Management and Its Role in Supporting Blood Supply

Richard R Gammon, Rounak Dubey, Gaurav K Gupta, Colleen Hinrichsen, Aikaj Jindal, Divjot Singh Lamba, Sadhana Mangwana, Amita Radhakrishnan Nair, Shaughn Nalezinski & Christopher Bocquet

Pages: 595-611

Paroxysmal Nocturnal Hemoglobinuria: Current Management, Unmet Needs, and Recommendations

Monika Oliver & Christopher J Patriquin

Pages: 613-628

Current Status and Challenges in Delivering Comprehensive Care for Patients with Hemophilia

Shosaku Nomura

Pages: 629-637

Hyperviscosity Syndrome Induced Bilateral Visual and Auditory Impairment in Therapy Resistant Waldenström Macroglobulinemia with MYD88 and CXCR4 Mutations

Marie M Plante, ErinMarie O Kimbrough, Amit K Agarwal, Liuyan Jiang, Kirk Bourgeois, Greta C Stamper, Michael W Stewart & Han W Tun

Pages: 639-648

Real-World Amount of Clotting Factor Concentrates Dispensed and Annual Medical Expenditures for Japanese Patients with Hemophilia B

Katsuyuki Fukutake, Kanae Togo, Linghua Xu, Leona E Markson, José Maria Jimenez Alvir, Ian Winburn & Toshiyuki Karumori

Pages: 649-661

Magnitude of Red Blood Cell Alloimmunization Among Pregnant Women Attending Antenatal Care at Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia

Tamirat Ersino Kebamo, Abinet Tantu Kombe, Temesgen Eticha, Mesay Arkew, Getachew Nigussie Bolado, Tadele Lankrew Ayalew, Kassahun Haile & Getachew Alemu Walano

Pages: 663-669

Predictors of Intensive Care Admission Among Adult Patients with Sickle Cell Disease in Eastern Province of Saudi Arabia

Mortadah Alsalman, Zaenb Alsalman, Hussain Abduljaleel Alkhalifa, Aman N Alfaraj, Ali Alkhalifah & Qasem Almulihi

Pages: 671-680

Assessment of Hematological Profiles and Prognostic Role of Hemogram-Derived Novel Markers for Diabetes Mellitus and Its Complications Among Type 2 Diabetes Mellitus Adult Patients Attending Bishoftu General Hospital, Central, Ethiopia: A Comparative Cross-Sectional Study

Dereje Abebe Regassa, Girum Tesfaye Kiya, Regassa Alemu Kebede & Woyesa Beyene

Pages: 681-699



Cerebral Venous Sinus Thrombosis induced By Hypercoagulation in Patient With Systemic Lupus Erythematosus: A Case Report and Literature Review

Lisda Amalia

To cite this article: Lisda Amalia (2023) Cerebral Venous Sinus Thrombosis induced By Hypercoagulation in Patient With Systemic Lupus Erythematosus: A Case Report and Literature Review, Journal of Blood Medicine, , 1-6, DOI: [10.2147/JBM.S387075](https://doi.org/10.2147/JBM.S387075)

To link to this article: <https://doi.org/10.2147/JBM.S387075>



© 2023 Amalia.



Published online: 10 Jan 2023.



Submit your article to this journal



Article views: 269



View related articles



View Crossmark data

Cerebral Venous Sinus Thrombosis induced By Hypercoagulation in Patient With Systemic Lupus Erythematosus: A Case Report and Literature Review

Lisda Amalia 

Department of Neurology, Faculty Medicine, Universitas Padjadjaran/RSUP Dr. Hasan Sadikin Bandung, Bandung, Indonesia

Correspondence: Lisda Amalia, Jl. Pasteur 38, Bandung, Indonesia, Email dr.lisda@gmail.com

Introduction: Systemic lupus erythematosus (SLE) are autoimmune diseases and cerebral venous sinus thrombosis (CVST) is coincidence regarding hypercoagulable condition of both diseases. The presence of both diseases in the same patient is rare, which suggests a relative incompatibility between these diseases.

Case Presentation: I report a female case with Systemic Lupus Erythematosus history, aged 27 years, with blurred vision, diplopia, severe headache, numbness and progressive right hemiparesis in 2 weeks. There was narrowing caliber at left transversus and right sigmoid sinus in magnetic resonance venography. She showed improvement in vision, numbness, headache and motor strength in right extremities after receiving pulse dose of corticosteroid for three days.

Conclusion: The distinction between SLE and CVST is a diagnostic challenge for the neurologist, and the presence of both diseases should be considered in patients with clinical neurologic manifestations who present with typical systemic manifestations of SLE and CVST. Neurogenic inflammation can induce disorders of the blood vessel wall (endothelium) that cause hypercoagulability and changes in acute vascular conditions can occur consisting of intraluminal platelet aggregation, thrombosis and also can cause total cerebral thrombotic venous or venular occlusion in SLE patients.

Keywords: cerebral venous sinus thrombosis, systemic lupus erythematosus, hypercoagulable state

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that is systemic in nature affecting the joints and several other organs including the skin, heart, lungs, kidneys and nervous system.^{1,2} The involvement of the central, peripheral, and autonomic nervous systems and psychiatric disorders in SLE is termed 'neuropsychiatric lupus' (NPSLE).^{3,4} Neuropsychiatric symptoms can also be one of the early manifestations of SLE. Research says that up to 40% of these symptoms appear during the first year after a diagnosis of SLE is made.⁵ The most common manifestation of SLE with Central Nervous System (CNS) involvement is headache, followed by mood disturbances and cognitive dysfunction, stroke and seizures.⁵⁻⁸

I discuss a case report of SLE with Cerebral Venous Sinus Thrombosis (CVST) induced by hypercoagulable condition. Written informed consent to publish case details and any accompanying images was provided by the patient. Dr. Hasan Sadikin General Hospital Bandung Human Research Ethics Committee approved this consent process and institutional approval was required to publish the case details.

Case Presentation

A 27-year-old female admitted to the emergency unit with blurred vision, diplopia, severe headache, numbness and progressive right hemiparesis in 2 weeks. She had history of Systemic Lupus Erythematosus (positive ANA profile) has

been recorded 5 years ago, and had routine medication before admission was mycophenolate sodium 360 mg every 8 hours and folic acid 5mg once daily.

On Examination

Fully alert and normal vital sign. Low visus on both eyes (20/200) with positive Relative Afferent Pupillary Defect (RAPD) and right abducen cranial nerve palsy. Right facial and hypoglossal nerve palsy, hemiparesis and hemihypesthesia on right extremities.

Laboratory Tests

WBCs $9.69 \times 10^3/\mu\text{L}$, RBC $4.18 \times 10^3/\mu\text{L}$, hemoglobin 10.60 (low) g/dL, platelet count $333 \times 10^3/\mu\text{L}$, neutrophils 94% (high), lymphocytes 5% (low), eosinophil 0%, basophil 5% and monocyte 1% (normal values: WBCs $4\text{--}10 \times 10^3/\mu\text{L}$, hemoglobin 12–15 g/dL, platelets count $140\text{--}450 \times 10^3/\mu\text{L}$, neutrophil 40–75%, lymphocytes 20–45%, monocytes 2–10%, eosinophils 2–6%, and basophils 0–1%). Prothrombin Time (PT) 13.30 seconds, aPTT 25.40 seconds, International Normalized Ratio (INR) 1.23 (high). D-dimer level 2.30 (high). Human Immunodeficiency Virus (HIV) antigen and Anti Phospholipid Antibodies was non-reactive. All electrolytes were in the normal limit. Liver and renal functions within the normal limit. Magnetic resonance venography showed narrowing caliber at left transversus and right sigmoid sinus (Figure 1). She was treated with intravenous methylprednisolone 500mg twice daily for three days, mycophenolate sodium 720 mg every 8 hours, warfarin 3 mg once daily, Mecobalamin 500mg every 8 hours, and Pregabalin 75mg twice daily for neuropathic pain symptoms.

After 7 days of hospitalization, she had improved the right limb's motor strength (walking with assistance), vision, headache, numbness and pain.

Discussion

The most common manifestation of SLE with Central Nervous System (CNS) involvement is headache, followed by mood disturbances and cognitive dysfunction, stroke and seizures.^{4,5}

Headache Mechanism in SLE: Neuroinflammation and Hypercoagulability

The mechanism of the pathogenesis of headache in Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) is not completely clear, there are several possible causes. Many autoantibodies have been detected in plasma samples of SLE patients and have been associated with NPSLE. Some of them are anti-ribosomal-P, anti-DNA/NR2, anti-DNA (16–1 idio type), antiphospholipid (aPL), anticardiolipin (aCL) and Gamma Ammino Butyric Acid (GABA) antibodies.^{3,5,6} It is hypothesized that auto-antibodies or pro-inflammatory cytokines circulating across the blood brain barrier (BBB) and entering the brain induce neurotoxicity.⁴ Hawro et al reported that in NPSLE patients, the presence of autoantibodies was significantly associated with headache, ischemia, stroke and seizures.^{4,12} The mechanisms that may be involved in the pathogenesis of the neuropsychiatric manifestations of SLE including headache in SLE patients are complex.^{12,14} Several studies suggest that there are several factors such as genetics, vascular damage and occlusion, BBB dysfunction, nerve damage mediated by autoantibodies or inflammatory mediators including cytokines, and also direct neuronal cell death.⁴ Two relevant pathogenetic pathways have been identified, namely the vascular-ischemic mechanism which is generally induced by aPL, immune complexes, and agglutination involving large and small blood vessels, which are considered to be more often responsible for the onset of focal neuropsychiatric symptoms.^{11,12,17} The second is an inflammatory neurotoxic process whose mechanism is predominantly mediated by complement activation, increased BBB permeability, migrating intrathecal autoantibodies and local production of immune complexes and pro-inflammatory cytokines and other inflammatory mediators, which may lead to diffuse neuropsychiatric manifestations, consider the causes of infection, especially herpes virus infection which can occur as a result of SLE immunosuppression.^{3,5,6,9,10}

Neuroinflammatory events involving activation of microglia and astrocytes also occur during the process of cortical spreading depression (CSD) which is the same pathophysiology of migraine with aura (MA).^{12,13} Several preclinical studies have revealed that CSD not only induces glial cell activation but also increases the expression of pro-inflammatory cytokines, adhesion and chemokines as well as the expression of toll-like receptors (TLR3 and TLR4).

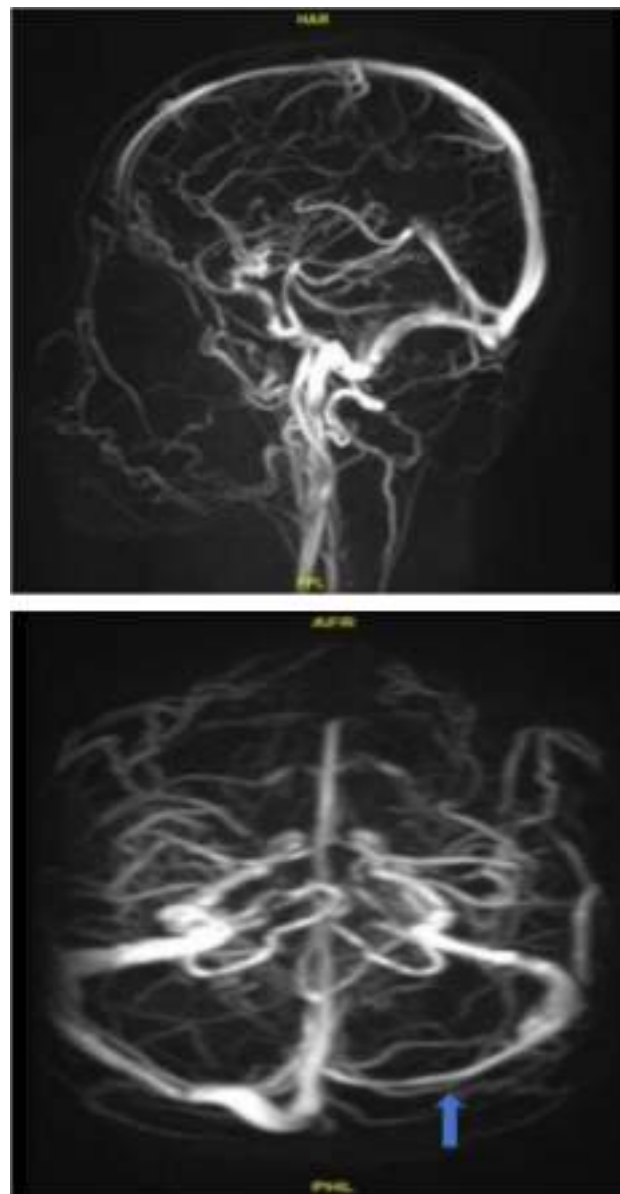


Figure 1 Magnetic Resonance Venography of this patient. There was a reduction in the caliber of the left transverse sinus and the right sigmoid sinus (blue arrow).

Neurogenic inflammation involving the dural and wattle vessels and also can induce disorders of the blood vessel wall (endothelium) that cause hypercoagulability.³¹ This causes several processes such as extravasation of plasma proteins due to increased meningeal vascular permeability and activation of immune cells, namely mast cells and macrophages.^{12–14}

Mast cell activation will trigger the formation of several pain mediators such as serotonin, histamine, heparin, proteases and arachidonic acid products, pro-inflammatory cytokines and chemokines that are involved in trigeminal peripheral sensitization.¹⁵ The C fibers of the trigeminal nerve will release calcitonin gene-related peptide (CGRP) which interacts with its own receptor on the dural vessels. This interaction will activate adenylate cyclase which causes dilation of blood vessels so that it will trigger pain.¹⁶ Astrocytes and glial cells also have CGRP receptors. The interaction of CGRP with its receptors on astrocytes and glia cells will induce the release of several pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β which dramatically amplify trigeminal nociception.^{16–18}

There is controversy over the pathogenesis of SLE and CVST involving autoimmunity.¹⁵ Several immunological mechanisms lead to inflammation of the blood vessel walls and an increase in the permeability of the blood-brain barrier.

In addition, perivascular leukocyte accumulation is associated with increased serum protein deposition. In addition, in some cases, changes in acute vascular conditions can occur consisting of intraluminal platelet aggregation and thrombosis and can cause total thrombotic venous or venular occlusion. In some cases, a severe inflammatory reaction has been observed in association with the veins.^{18,25} Impaired blood flow due to blockage of the venous system will cause increased pressure on brain tissue, because the venous system is blocked in the dural sinus area. Increased pressure on brain tissue will cause brain edema around the blockage area of the venous system. Furthermore, the capillaries and arterioles will rupture and cause cerebral hemorrhage, if the pressure increases higher, the bleeding can spread to the nearest subarachnoid space causing decreased consciousness to death.³¹

In this patient there is thrombosis in the vein (CVST) which can be caused by hypercoagulable conditions (elevated of D dimer level) and will cause blockage of venous system resulting in increased intracranial pressure (benign intracranial hypertension). The complex interaction between endothelial cells and Lupus Antibody (LA) will trigger inhibition of proteins C and S that can cause hypercoagulable state which are responsible for the occurrence of thrombosis.²⁵ Impaired fibrinolysis, antithrombin III, hyperfibrinemia, or changes in coagulation may also lead to thrombosis.^{14,15,25}

The presence of optic neuropathy and MRI images in the form of ovoid lesions with hyperintense T2 in the parietal and occipital subcortical areas accompanied by bilateral optic neuritis images suggest a demyelination process (multiple sclerosis) which is still possible to diagnose in comparison with SLE lesions.^{19,20} In addition, optic neuropathy in SLE can also be caused by an ischemic process. Fluorescein angiography can help differentiate between some cases of optic neuritis and ischemic optic neuropathy, which often results from thrombosis or vasculitis.^{9,12} It is also important to consider the cause of infection, particularly herpes virus infection, which can occur as a result of SLE immunosuppression.^{17,18}

Management of SLE with CVST

Adrenocorticotrophic (ACTH) hormone gel was approved by the United States Food and Drug Administration as a treatment option for SLE in 1952.^{23,24,29,30} ACTH has anti-inflammatory and immunomodulatory effects due to activation of central and peripheral melanocortin receptors.³⁰ Regarding SLE patients with moderate or severe active SLE, an open-label study showed that ACTH gel may provide significant disease activity reduction.^{29,30}

However, there is controversy regarding the administration of high-dose steroids in CVST patients. The use of steroids is associated with coagulation status so that it can affect the CVST condition. Patients with chronic inflammatory disease are at risk for venous thromboembolism (VTE).²⁵ The risk of developing VTE is related to an exacerbation of the disease usually treated with corticosteroids.²⁶ When corticosteroids induce a procoagulant state, patients with exacerbations and receiving corticosteroid therapy have a very high risk of developing VTE. Hypercoagulability induced by steroid therapy is associated with elevated factors VII, VIII, and XI. There are studies that show an increase in FVIII levels after short-term administration of high-dose dexamethasone in healthy men, which is in line with observations in patients with Cushing's syndrome. However, other non-genomic studies have shown that high doses of acute glucocorticoids increase activation of endothelial nitric oxide synthase (eNOS), which may inhibit VWF secretion. While endothelial dysfunction and increased oxidative stress and chronic insulin resistance associated with excess glucocorticoid in the long term have been reported to increase plasma VWF levels. It is also explained that the long-term use of oral corticosteroids allows for an increase in factors II, V, VII, IX, X, XII and fibrinogen which further induces a hypercoagulable state.^{21,22,25,26}

In addition, the presence of steroid therapy in SLE can interact with anticoagulant therapy, namely warfarin which is used as CVST therapy.²² There are studies showing a supratherapeutic effect of INR in individuals taking warfarin and steroids. The exact mechanism of interaction between warfarin and oral corticosteroids is not clearly known, but it is thought to be related to the process of both drugs in the liver. Methylprednisolone, prednisone, and warfarin are metabolized in the liver via the CYP3A4 isoenzyme pathway.²⁶ Inhibition of warfarin metabolism potentially occurs as a result of competitive binding to CYP3A4. In addition to the CYP3A4 pathway, warfarin is also a substrate for the CYP1A2, 2D6, and 2C9 isoenzyme pathways. However, there is another influence, namely genetic deficiency of the cytochrome P450 isoenzyme that causes various compensatory warfarin metabolism through different isoenzyme pathways. Kaufman says another possible theory of warfarin's effect with steroids is related to serum pH. The use of steroids

will increase the serum pH which can cause the binding of warfarin to protein to decrease so that it will increase the free warfarin level in the blood.^{26,27}

In this patient was treated with intravenous methylprednisolone 500mg twice daily for three days, mycophenolate sodium 720 mg every 8 hours, warfarin 3 mg once daily, Mecobalamin 500mg every 8 hours, and Pregabalin 75mg twice daily for neuropathic pain symptoms. After 7 days of hospitalization, she had improved the right limb's motor strength (walking with assistance), vision, headache, numbness and pain. It needs to be watched out for in relation to the possibility of bleeding side effects from warfarin use.^{25,26}

Limitation of Study

This article had several limitations. I did not assess the panel of pro-inflammatory assay in this patient.

Conclusion

The distinction between SLE and cerebral venous sinus thrombosis is a diagnostic challenge for the neurologist, and the presence of both diseases should be considered in patients with clinical neurologic manifestations of SLE who present with headache and neurological deficit. Neurogenic inflammation can induce disorders of the blood vessel wall (endothelium) that cause hypercoagulability and changes in acute vascular conditions can occur consisting of intraluminal platelet aggregation, thrombosis and also can cause total thrombotic venous or venular occlusion in cerebral of SLE patients. Health-care professionals, involved clinicians, especially neurologist, should be informed to with this complication, addressing a prompt diagnosis and proper treatment to decrease the CVST-related morbidity and public health burden in SLE patients.

Disclosure

The author reports no conflicts of interest in this work.

References

1. Ahn GY, Kim D, Won S, et al. Prevalence, risk factors, and impact on mortality of neuropsychiatric lupus: a prospective, single-center study. *Lupus*. 2018;27(8):1338–1347.
2. Zandman-Goddard G, Chapman J, Shoenfeld Y. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. *Semin Arthritis Rheum*. 2007;36(5):297–315. doi:10.1016/j.semarthrit.2006.11.003
3. Yoshio T, Okamoto H, Hirohata S, Minota S. IgG anti-NR2 glutamate receptor autoantibodies from patients with systemic lupus erythematosus activate endothelial cells. *Arthritis Rheum*. 2013;65(2):457–463. doi:10.1002/art.37745
4. Duarte-Delgado NP, Vázquez G, Ortiz-Reyes BL. Blood-brain barrier disruption and neuroinflammation as pathophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus. *Autoimmun Rev*. 2019;18(4):426–432. doi:10.1016/j.autrev.2018.12.004
5. Horwitz H, Friis T, Modvig S, et al. Differential diagnoses to MS: experiences from an optic neuritis clinic. *J Neurol*. 2014;261:98–100.
6. Optic Neuritis Study Group. Visual function 15 years after optic neuritis: a final follow-up report from the optic neuritis treatment trial. *Ophthalmology*. 2008;115:1079–82.e5.
7. Pérez Bartolomé F, García Vasco L, Abreu ventura N, et al. Diagnosis approach of optic neuritis. *J Neurol Neurophysiol*. 2015;6:345.
8. Vollmer T, Signorovitch J, Huynh L, et al. The natural history of brain volume loss among patients with multiple sclerosis: a systematic literature review and meta-analysis. *J Neurol Sci*. 2015;357(1–2):8–18.
9. Goldenberg MM. Multiple sclerosis review. *P T*. 2012;37(3):175–184.
10. Yetimlar Y, Seçil Y, Inceoglu AK, et al. Unusual primary manifestations of multiple sclerosis. *N Z Med J*. 2008;121:47–59.
11. Ergun U, Özer G, Şekercan S, et al. Headaches in the different phases of relapsing-remitting multiple sclerosis A tendency for stabbing headaches during relapses. *Neurologist*. 2009;15:212–216.
12. Gee JR, Chang J, Dublin AB, Vijayan N. The association of brainstem lesions with migraine- like headache: an imaging study of multiple sclerosis. *Headache*. 2005;45:670–677.
13. Elliott DG. Migraine in multiple sclerosis. *Int Rev Neurobiol*. 2007;79:281–302.
14. Appenzeller S, Costallat LT. Clinical implications of migraine in systemic lupus erythematosus: relation to cumulative organ damage. *Cephalalgia*. 2004;24(12):1024–1030. doi:10.1111/j.1468-2982.2004.00785.x
15. Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. *A Rev Immunol*. 1992;10:153–187.
16. Bainbridge JL, Corboy JR, Gidal BE, et al. Multiple sclerosis. In: DiPiro JT, Talbert RL, Yee GC, editors. *Pharmacotherapy: A Pathophysiological Approach*, 5th. New York, NY: McGraw-Hill; 2002:1019–1030.
17. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis. *Lancet Neurol*. 2017. doi:10.1016/S1474-4422(17)30470-2
18. Berkovich RR. Acute multiple sclerosis relapse. *Continuum*. 2016;22:799–814.
19. Kim HJ, Paul F, Lana-Peixoto MA, et al. MRI characteristics of neuromyelitis optica spectrum disorder: an international update. *Neurology*. 2015;84:1165–1173.
20. Aliaga ES. MRI mimics of multiple sclerosis. *Handb Clin Neurol*. 2014;122:291–316.

21. Sorensen PS, Haas J, Sellebjerg F, et al. IV immunoglobulins as add-on treatment to methylprednisolone for acute relapses in MS. *Neurology*. 2004;63(11):2028–2033.
22. Ciccone A, Beretta S, Brusaferrì F, Galea I, Protti A, Spreafico C. Corticosteroids for the long-term treatment in multiple sclerosis. *Cochrane Database Syst Rev*. 2008. doi:10.1002/14651858.CD006264
23. Costello K, Halper J, Kalb R, Skutnik L, Genzyme S, Rapp R The use of modifying drug therapy in multiple sclerosis. 2019.
24. Yamout B, Sahraian M, Bohlega S, et al. Consensus recommendations for the diagnosis and treatment of multiple sclerosis: 2019 revisions to the MENACTRIMS guidelines. *Mult Scler Relat Disord*. 2020;37:101459.
25. Levi M, van der Poll T. Inflammation and coagulation. *Crit Care Med*. 2010;38:S26–S34.
26. Costedoat-Chalumeau N, Amoura Z, Aymard G, et al. Potentiation of vitamin K antagonists by high-dose intravenous methylprednisolone. *Ann Intern Med*. 2000;132:631–635.
27. Harrison DM, Gladstone DE, Hammond E, et al. of relapsing–remitting multiple sclerosis with high-dose cyclophosphamide induction followed by glatiramer acetate maintenance. *Mult Scler*. 2012;18:202–209.
28. Michel L, Vukusic S, De Seze J, et al. Mycophenolate mofetil in multiple sclerosis: a multicentre retrospective study on 344 patients. *J Neurol Neurosurg Psychiatry*. 2014;85:279–283.
29. Massacesi L, Tramacere I, Amoroso S, et al. Azathioprine versus beta interferons for relapsing–remitting multiple sclerosis: a multicentre randomized non-inferiority trial. *PLoS One*. 2014;9:e113371.
30. Fiechtner JJ, Montroy T. Treatment of moderately to severely active systemic lupus erythematosus with adrenocorticotrophic hormone: a single-site, open-label trial. *Lupus*. 2014;23:905–912.
31. Amalia L. The role of platelet-selectin as a marker of thrombocyte aggregation on cerebral sinus venous thrombosis. *J Blood Med*. 2022;13:267–274.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Role of Hemin in the Immune Response of T Follicular Helper Lymphocytes Expressing T-Cell Immunoreceptor with Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Domains, Programmed Cell Death-1, and Interleukin-21 in Allo-Auto Positive and Negative Thalassemia

Betty Agustina Tambunan, I Dewa Gede Ugrasena & Aryati

To cite this article: Betty Agustina Tambunan, I Dewa Gede Ugrasena & Aryati (2023) Role of Hemin in the Immune Response of T Follicular Helper Lymphocytes Expressing T-Cell Immunoreceptor with Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Domains, Programmed Cell Death-1, and Interleukin-21 in Allo-Auto Positive and Negative Thalassemia, *Journal of Blood Medicine*, , 7-17, DOI: [10.2147/JBM.S393134](https://doi.org/10.2147/JBM.S393134)

To link to this article: <https://doi.org/10.2147/JBM.S393134>



© 2023 Tambunan et al.



Published online: 13 Jan 2023.



Submit your article to this journal



Article views: 318



View related articles



View Crossmark data

Role of Hemin in the Immune Response of T Follicular Helper Lymphocytes Expressing T-Cell Immunoreceptor with Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Domains, Programmed Cell Death-1, and Interleukin-21 in Allo-Auto Positive and Negative Thalassemia

Betty Agustina Tambunan¹⁻³, I Dewa Gede Ugrasena^{3,4}, Aryati^{2,3}

¹Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; ²Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; ³Dr. Soetomo General Academic Hospital, Surabaya, Indonesia; ⁴Department of Pediatrics, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Correspondence: Aryati, Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, St. Mayjen. Prof. Dr. Moestopo 47, Surabaya, East Java, 60132, Indonesia, Tel +6281230570493; +62-031-5020251, Email aryati@fk.unair.ac.id

Introduction: Repeated transfusions in thalassemia patients can cause several complications, including alloimmunization and autoimmunization.

Purpose: This study compares the immune response of T follicular helper (Tfh) lymphocytes expressing T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory domains (TIGIT), programmed cell death-1 (PD-1), and interleukin-21 (IL-21) between patients with allo-auto positive and negative thalassemia before and after hemin administration.

Materials and Methods: This study used a quasi-experimental pre- and post-test design and was performed between April and November 2021 at the Dr. Soetomo General Academic Hospital in Surabaya, Indonesia. It enrolled 29 patients with allo-auto positive thalassemia and 28 with allo-auto negative, and 9 mL of whole blood (WB) was drawn from each patient. Hemin solution (20 µM) was added to 5 mL of WB, incubated for two hours, processed into peripheral blood mononuclear cells (PBMCs) in RPMI media, and cultured with 5% CO₂ for three days. The 4 mL WB sample was also processed into PBMCs. PBMC cells cultured and without cultured were examined by flow cytometry using a BD FACSCalibur after surface and intracellular staining. Differences in Tfh cells expressing TIGIT, PD-1, and IL-21 between thalassemia groups before and after hemin administration were compared using independent *t*-tests or Mann-Whitney *U*-tests (*p* < 0.05).

Results: Tfh cell expression did not differ between groups before hemin administration and increased after hemin administration. The increase in Tfh cell expression was higher in the allo-auto positive group. TIGIT and PD-1 expression in Tfh cells did not differ between groups, but TIGIT decreased after hemin administration in contrast to PD-1 result. IL-21 expression in Tfh cells did not differ between groups and did not change after hemin administration.

Conclusion: Hemin affected the expression of Tfh cells in both group thalassemia, but there was no difference of Tfh cell expression between the groups.

Keywords: thalassemia disease, T follicular helper, TIGIT, PD-1, interleukin-21

Introduction

Thalassemia is an autosomal recessive hereditary disease. A partial or complete deficiency of globin chain tetramers in thalassemia causes chronic hemolytic anemia. The Thalassemia International Federation classifies thalassemia into transfusion-independent and transfusion-dependent based on its need for transfusion.¹ As technology advances, disease-causing mutations have become treatment targets. Several approaches have been explored as nucleic acid-based therapies. However, several limitations challenge their implementation, such as stability, delivery method, suitable vector, and optimal nucleic acid dose.²

Transfusion remains the primary treatment recommended for thalassemia. However, repeated transfusions can cause iron overload, characterized by increased serum ferritin levels that interfere with 25(OH)D synthesis in the liver.³ High excess iron also causes impaired glucose metabolism.⁴ Other complications are common, such as mild depression, especially in patients with high serum cortisol levels,⁵ transfusion reactions, infection, alloimmunization, and autoimmunization.⁶ Alloimmunization rates are reported to be 5–30% in thalassemia major in Mediterranean and Southeast Asian countries. A Thai study found an alloimmunization rate of 17.5% in transfusion-dependent thalassemia patients.⁷ Research on patients with homozygous beta-thalassemia who received repeated transfusions found that 87 of 385 patients (22.6%) experienced autoimmunization.⁸ The reported autoimmunization rates in thalassemia patients in several countries include 28.8% in Egypt,⁹ 23% in Hong Kong,¹⁰ 22.8% in Albania,¹¹ and 6.5% in the US.¹²

Alloimmunization and autoimmunization can have many disadvantages, including acute and delayed hemolytic transfusion reactions, difficulty in obtaining compatible blood leading to delayed transfusions, additional pre-transfusion tests, and increased costs for laboratory tests and patient management.⁶ Therefore, the role of the immune response in alloimmunization and autoimmunization incidence in thalassemia patients must be further studied.¹³

Several studies on the immune response in chronic hemolytic conditions have been performed in sickle cell disease (SCD) patients. Cell culture studies of SCD patients who received repeated transfusions showed an increased response of CD4+ T cells to the hemin administration in alloimmunized patients compared to those without alloimmunization.¹⁴ Hemin is a marker of red blood cell degradation products that induce changes in TCD4+ cell subsets through activation of heme oxygenase-1 (HO-1).¹⁵ Another study on SCD patients showed that T follicular helper (Tfh) cells expressing programmed cell death-1 (PD-1) are more numerous in patients without alloimmunization compared to those with alloimmunization, while interleukin-21 (IL-21) expressing cells were more numerous in patients with alloimmunization.¹⁶ Cell culture studies of SCD patients with repeated transfusions showed an increase in Tfh cells expressing T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory domains (TIGIT) in alloimmunized versus non-alloimmunized subjects, while Tfh cells expressing PD-1 were not different.¹⁷

Whether the immune response in thalassemia is similar to SCD patients remains unknown.¹⁸ Since chronic hemolysis occurs in both SCD and thalassemia patients, this study compares the immune response of Tfh lymphocytes expressing TIGIT, PD-1, and IL-21 between transfusion-dependent thalassemia patients with (allo-auto positive) and without (allo-auto negative) alloimmunization and autoimmunization before and after hemin administration. The study's results are expected to provide information for therapy development in thalassemia patients through modification of the immune response of Tfh cells, especially in patients with alloimmunization.

Materials and Methods

This study was quasi-experimental with a non-equivalent control group and pre- and post-test design. It was performed between April and November of 2021 at the Dr. Soetomo General Academic Hospital in Surabaya, Indonesia. This study was approved by Local Institutional Human Research Ethics Committee at the Dr. Soetomo General Academic Hospital (no. 0527/LOE/301.4.2/VII/2021) in accordance with the standards laid down in the 1964 Declaration of Helsinki.

We enrolled 29 patients with transfusion-dependent allo-auto positive thalassemia and 28 with allo-auto negative thalassemia. Sample size calculated by determining the sample size for experimental research with two groups

(1- β =0.80). Patients underwent standard set blood sample drawing after obtaining informed consent. Informed consent was obtained from parents or legal guardians for research participants under 18 years old. Heparin blood samples were taken from each patient. In particular, 9 mL of whole blood was drawn from each patient and divided into two heparinized tubes; one contained 5 mL for culture and the other contained 4 mL for non-culture. A 20 μ M hemin solution was added to culture samples, incubated for two hours, and prepared to manufacture peripheral blood mononuclear cells (PBMCs).

PBMC cells were diluted to a density of 10^6 cells/mL and divided into three 1 mL tubes. Fetal bovine serum, streptomycin, and penicillin were added to each tube and mixed. Next, they were placed in an incubator with 5% CO₂ for three days. PBMC cultures were harvested after three days and centrifuged at 1500 rpm for 10 minutes to separate PBMCs from the supernatant. PBMCs were washed twice and prepared for flow cytometry. The 4 mL blood samples were added to 4 mL of PBS, mixed, divided into two tubes, and prepared for PBMC manufacturing.

Tfh lymphocytes expressing TIGIT, PD-1, and IL-21 were identified using flow cytometry with a BD FACS Calibur instrument. The Tfh cells were identified by surface staining by adding mouse anti-human CD4 labeled with fluorescein isothiocyanate (BD Pharmingen) and rat anti-human C-X-C motif chemokine receptor 5 (CXCR5/CD185) labeled with PerCP-Cy5.5 (BD Pharmingen). Monoclonal anti-TIGIT labeled with allophycocyanin (eBioscience) as the TIGIT surface marker. Monoclonal mouse anti-human PD-1 (CD279) antibody (BD Pharmingen) labeled with phycoerythrin was added as the PD-1 surface marker. Intracellular staining was performed by adding mouse anti-human IL-21 labeled with Alexa Fluor 647 (BD Pharmingen).

Statistical Analysis

The differences in Tfh cells expressing TIGIT, PD-1, and IL-21 between allo-auto positive and negative thalassemia groups before and after hemin administration were assessed with independent *t*-tests when normally distributed and with Mann–Whitney *U*-tests when nonnormally distributed. All results with *p* < 0.05 were considered statistically significant and analyzed using IBM SPSS Statistics Version 21.

Results

Participants' demographic data (gender, age, and blood type) were collected when they first received blood transfusions. The allo-auto negative thalassemia group was dominated by the O blood type (50%), while the allo-auto positive thalassemia group was dominated by the B blood type (44.8%). All patients were rhesus positive (Table 1).

Table 1 Participant Characteristics

Variable	Allo-Auto Negative Thalassemia	Allo-Auto Positive Thalassemia
Sex		
Male	14 (50.0%)	8 (27.6%)
Female	14 (50.0%)	21 (72.4%)
Age (years)		
1–5	3 (10.7%)	2 (6.9%)
6–10	8 (28.6%)	4 (13.8%)
11–17	9 (32.1%)	10 (34.5%)
Adult (18–39)	8 (28.6%)	13 (44.8%)
Blood group		
A	7 (25.0%)	7 (24.1%)
B	5 (17.9%)	13 (44.8%)
O	14 (50.0%)	8 (27.6%)
AB	2 (7.1%)	1 (3.4%)
Rh positive	28 (100.0%)	29 (100.0%)

Note: *p* significant at $\alpha = 0.05$ (Mann–Whitney test).

Table 2 Antibody and Autocontrol Screening Results

Antibody Screening	Autocontrol	Alloimmunization/Autoimmunization	Total
Negative	Positive	Autoimmunization	13 (44.8%)
Positive	Negative	Alloimmunization	4 (13.8%)
Positive	Positive	Alloimmunization + autoimmunization	12 (41.4%)

Of the 29 allo-auto positive thalassemia patients, 13 showed positive autocontrol and negative antibody screening (44.8%), termed thalassemia with autoimmunization. Negative autocontrol and positive antibody screening were found in four patients (13.8%), termed alloimmunization thalassemia. Positive autocontrol and positive antibody screening were found in 12 patients (41.4%), termed alloimmunization and autoimmunization thalassemia (Table 2).

T Follicular Helper Cells

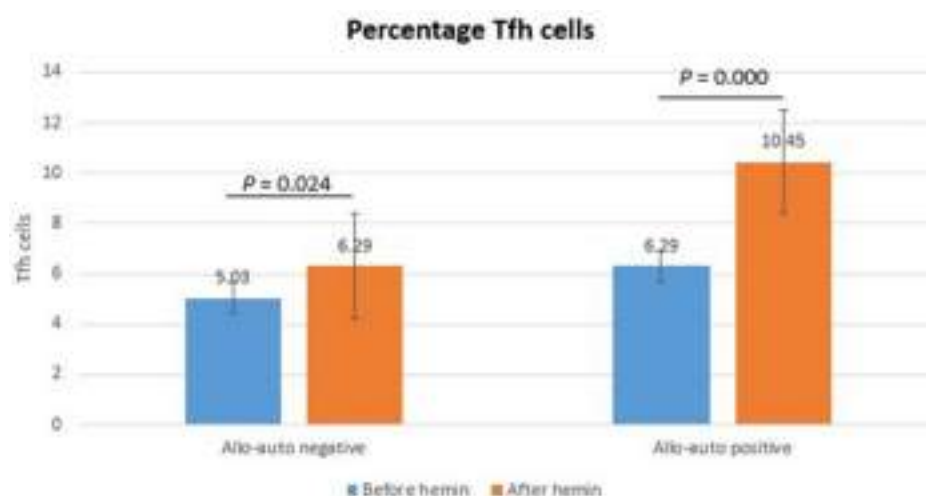
Tfh cells in allo-auto negative and positive thalassemia significantly increased after hemin administration ($p = 0.024$ and $p = 0.000$, respectively). In addition, the increase in Tfh cells differed significantly between groups ($p = 0.030$). Tfh cell numbers in allo-auto negative thalassemia were not significantly different from allo-auto positive thalassemia before hemin administration ($p = 0.267$) but were significantly higher after hemin administration ($p = 0.024$) (Figure 1).

Activation of Tfh Cells Expressing TIGIT

Tfh cells expressing TIGIT in allo-auto negative and positive thalassemia significantly decreased after hemin administration ($p = 0.000$ and $p = 0.001$, respectively). The change in Tfh cells expressing TIGIT did not differ significantly between groups ($p = 0.326$). Tfh cells expressing TIGIT did not differ significantly between groups before ($p = 0.083$) or after ($p = 0.199$) hemin administration (Figure 2).

Tfh Cells Expressing PD-1

Tfh cells expressing PD-1 did not differ significantly between allo-auto negative and positive thalassemia before ($p = 0.835$) and after ($p = 0.133$) hemin administration. However, Tfh cells expressing PD-1 significantly increased after hemin administration in allo-auto negative ($p = 0.000$) and allo-auto positive ($p = 0.001$)

**Figure 1** T follicular helper (Tfh) cells.

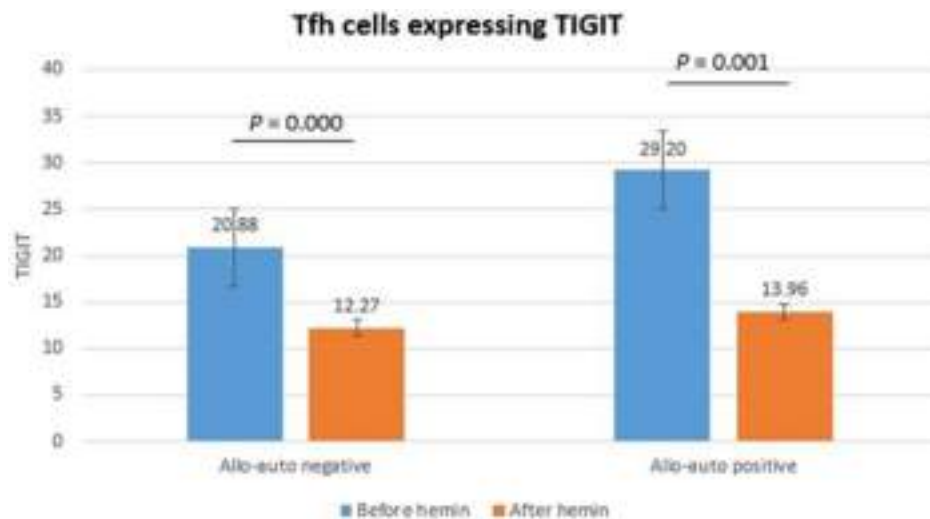


Figure 2 Tfh cells expressing TIGIT.

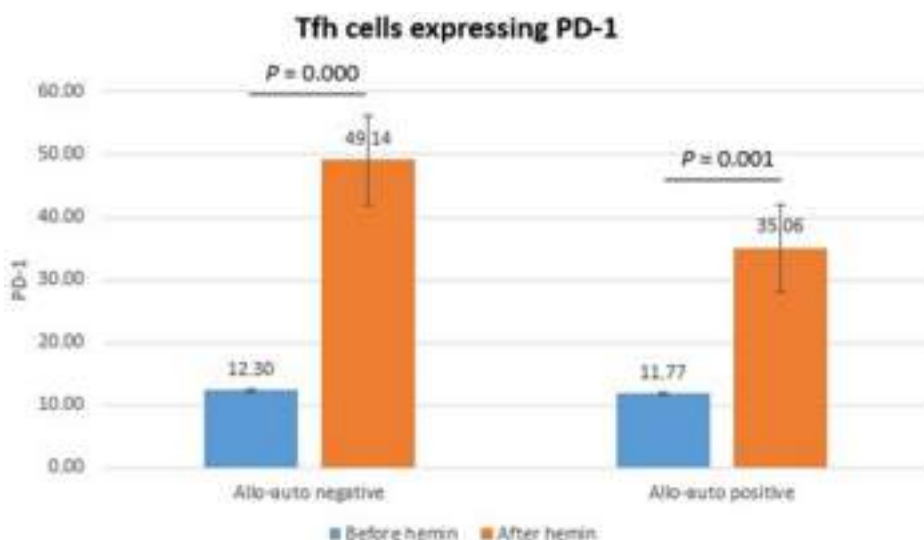


Figure 3 Tfh cells expressing PD-1.

thalassemia. However, the change of Tfh cells expressing PD-1 did not differ significantly between groups ($p = 0.238$) (Figure 3).

Tfh Cells Expressing IL-21

Tfh cells expressing IL-21 did not differ significantly between allo-auto negative and positive thalassemia before ($p = 0.914$) and after ($p = 0.195$) hemin administration. Tfh cells expressing IL-21 did not differ significantly after hemin administration in allo-auto negative ($p = 0.995$) and positive ($p = 0.063$) thalassemia. The change in Tfh cells expressing IL-21 did not differ significantly between groups ($p = 0.513$) (Figure 4).

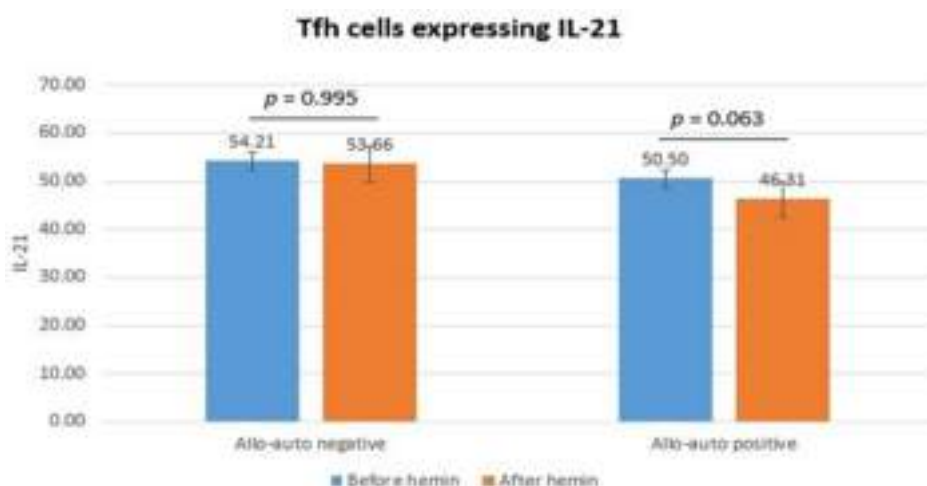


Figure 4 Tfh cells expressing IL-21.

Discussion

Fifty-seven transfusion-dependent thalassemia patients were included in this study, 28 with allo-auto negative and 29 with allo-auto positive thalassemia. Our results suggest that the allo-auto positive thalassemia group was dominated by 13 autoimmunization patients (44.8%), followed by 12 alloimmunization and autoimmunization patients (41.4%), and four alloimmunized patients (13.8%). An Albanian prospective cohort study followed 118 thalassemia patients receiving regular transfusions for five years, screened for antibodies, and given blood with matched ABO, rhesus, and Kell blood group systems. Of these 118 patients, 14 (11.8%) were alloimmunized and 27 (22.8%) were autoimmunization. They found that of the 14 alloimmunized patients, 11 also had autoimmunization.¹¹

An Egyptian study found that of 200 thalassemia patients receiving repeated transfusions, those with alloimmunization were more alloimmunized (36; 18%) than autoimmunized (33; 16.5%).¹⁸ A Chinese study reported that of their 382 patients, 294 (77.0%) were antibody negative and 88 (23.0%) were antibody positive. Of the antibody-positive patients, 70 (79.5%) showed alloimmunization and 18 (20.5%) showed autoimmunization.¹⁰ Our study is consistent with the Albanian study, suggesting that many patients with alloimmunization also have autoimmunization. The frequency of patients with autoimmunization is higher than with alloimmunization, which is inconsistent with the Egyptian and Chinese studies. The influencing factors in the frequency of alloimmunization and autoimmunization are unclear. Overall, our study is consistent with all three studies because thalassemia patients experienced unexplainable alloimmunization or autoimmunization.

Our study found that allo-auto negative thalassemia is dominated by blood type O, while allo-auto positive thalassemia is dominated by blood type B. A Greek study of 65 thalassemia patients receiving repeated transfusions found that 36.9% had blood group B and were rhesus positive.¹⁸ An Indian study of 500 thalassemia patients receiving repeated transfusions found that 36.8% had blood group B and were rhesus positive.¹⁹ Unfortunately, these two studies did not classify thalassemia as with or without alloimmunization. Whether there is a relationship between the incidence of alloimmunization and autoimmunization with the ABO blood group remains unclear.

Tfh cells in allo-auto negative thalassemia were not significantly different from allo-auto positive thalassemia before hemin administration. Tfh cells are a subset of CD4+ T cells that stimulate B cells to produce antibodies in the effective humoral immune response.²⁰ Tfh cells were characterized by their expression of the transcription factor nuclear transcriptional repressor B cell lymphoma 6 (BCL6), the CXCR5, inducible co-stimulator (ICOS), PD-1, and IL-21.²¹

Since 2011, CD4+ CXCR5+ T cells in peripheral blood have been known as circulating Tfh cells, which have been shown to promote B cell differentiation into plasma cells and produce immunoglobulin G (IgG) in vitro.²² Elevated Tfh cells have been found in patients with systemic lupus erythematosus (SLE), myasthenia gravis, and juvenile

dermatomyositis.²³ Excessive activation of Tfh cells can also lead to autoimmunization.²⁴ Other studies have also shown an increase in circulating Tfh cells in patients with active autoimmune disease.²⁵

Circulating Tfh cells account for about 6% of the total CD4⁺ T cells in peripheral blood in healthy individuals.²² Tfh cells have antigen-specific memory and regulate memory B cells.²⁶ Our study found that the median percentage of Tfh cells from the CD4⁺ T cells in allo-auto negative thalassemia patients was lower (5.03%) than in allo-auto positive thalassemia patients (6.29%). However, the percentage of Tfh cells did not differ significantly between groups. Another study found that the percentage of Tfh cells in SCD patients was not significantly different between those without alloimmunization and those with alloimmunization.¹⁷

A study in mice modeled autoimmune hemolytic anemia (AIHA) and showed an increase in the number of Tfh cells and the ratio of Tfh to T follicular regulatory T cells (Tfr) in mice with positive autoantibodies. They also found that Tfh cells injected into AIHA-modeled mice stimulated the formation of autoantibodies, indicating the important role of Tfh cells in controlling the induction of B cells to produce antibodies in AIHA. Increased Tfh cell activity is associated with a response to exposure to a given red cell antigen. Weekly intraperitoneal injections of red blood cells in rats led to the formation of autoantibodies against erythrocytes five to six weeks after immunization. Peak erythrocyte autoantibody levels were formed within 10–12 weeks post-injection. They correlated with an increase in reticulocytes and significantly reduced the lifespan of red blood cells. The increase in Tfh cells correlated positively with anti-RBC IgG levels.²¹

Hemin administration increased the number of Tfh cells in both groups. Tfh cells were higher in allo-auto positive thalassemia patients after hemin administration. Hemoglobin and heme resulting from the destruction of red blood cells in thalassemia hemolytic anemia can act as erythrocytic danger-associated molecular pattern molecules that activate the innate (natural) immune response.²⁷ Heme induces T regulatory (Treg) cell proliferation and inhibits Th1 through HO-1 activation in SCD patients without alloimmunization but not in patients with alloimmunization.²⁸ Hemin administration was believed to induce Tfh cell proliferation in both groups due to the Tfh response to heme. Hemin induced a stronger Tfh cell response in allo-auto positive thalassemia patients, indicated by a larger increase in the percentage of Tfh cells. We hypothesize that the increase in Tfh cells in allo-auto positive thalassemia patients might be related to Tfh cells, which are more sensitive to hemin exposure due to their memory of specific red blood cell antigens contacted.

Tfh cells expressing TIGIT in allo-auto negative thalassemia patients were not significantly different from allo-auto positive thalassemia patients. Another study of healthy individuals showed that the percentage of Tfh cells expressing TIGIT in peripheral blood was $47\% \pm 3.2\%$.¹⁷ Our study showed that the median percentage of Tfh cells expressing TIGIT in peripheral blood of allo-auto negative thalassemia patients was lower than in healthy individuals in Godefroy et al but with a wider range.¹⁷ The highest percentage was almost the same as normal individuals (20.88%; 4.93–47.29%) and allo-auto positive thalassemia patients (29.20%; 4.23–51.55%). Our study showed that the percentage of Tfh cells expressing TIGIT did not differ significantly between groups, consistent with the SCD study that showed the percentage of Tfh cells expressing TIGIT did not differ significantly between patients with allo-auto positive and negative thalassemia.

A significant decrease in Tfh cells expressing TIGIT in both groups after hemin administration was showed in our study. Studies of autoimmune SLE patients showed that they have more Tfh cells expressing TIGIT than healthy individuals, correlating with severity as assessed by the SLE disease activity index criteria.²⁹ Cancer research shows that TIGIT inhibition in Treg cells (CD4⁺ FOXP3⁺ TIGIT⁺) increases the activity of TCD8⁺ and NK cells, inhibiting tumor growth.³⁰ The role of Tfh cells in thalassemia remains unclear. Our study showed that hemin administration to PBMCs from thalassemia patients decreased Tfh cells expressing TIGIT. The decrease in the percentage of Tfh cells expressing TIGIT after hemin administration might be an anti-inflammatory response to HO-1 activation. This hypothesis is consistent with the effect of hemin on cytokine regulation through the activation of HO-1, which has an anti-inflammatory effect by inhibiting macrophages secreting proinflammatory cytokines (interleukin-1 β and tumor necrosis factor α) and inducing the release of interleukin-10.³¹

Another study of SLE patients showed a decrease in Tfh cells expressing TIGIT after stimulation with mitogen phytohemagglutinin. They also used recombinant CD155 (TIGIT receptors on dendritic cells), which decreased CD4⁺ T cell proliferation. This finding suggests that TIGIT may act as a negative regulator of TCD4⁺ cell activity.²⁹ HO-1 acts as an immunosuppressant, inhibiting dendritic cell maturation.³² This study proposed that hemin might activate HO-1,

inhibiting the response of dendritic cells to TIGIT and decreasing the percentage of Tfh cells expressing TIGIT after hemin administration.

Tfh cells expressing PD-1 did not differ significantly between groups before and after hemin administration. The median percentage of Tfh cells expressing PD-1 in our study for allo-auto negative and positive thalassemia patients was lower but broader than in healthy individuals in another study.³³ Our study also found that Tfh cells expressing PD-1 increased significantly in both groups after hemin administration. The median percentage of Tfh cells expressing PD-1 result was lower than in lymphoma patients from another study.³³

PD-1 is a co-inhibitor of cytokine production and effector T cell proliferation. This mechanism maintains tolerance to autoantigens and prevents autoimmunity.³⁴ Inhibition of T-cell receptor signaling and costimulatory CD28 is considered the main pathway of PD-1 action.³⁵ Programmed death-ligand 1 (PD-L1) is expressed by hematopoietic and nonhematopoietic cells. Cells expressing PD-1 will bind to PD-L1, inducing signals that inhibit the function and survival of Tfh cells expressing PD-1.³⁶ Another study found that PD-1 inhibits the function of Tfr. PD-1 deficiency in Tfr cells in the peripheral blood increases suppressive capacity.³⁷ In-vitro studies of T cells in the lymph nodes of patients infected with the human immunodeficiency virus (HIV) showed an increase in Tfh cells expressing PD-1 followed by a decrease in ICOS expression and IL-21 secretion, suggesting that PD-1 inhibits the increased production of HIV-specific immunoglobulins.²⁰

Experimental animal studies have shown that dendritic cells are required to initiate the transformation of naive TCD4 cells into Tfh cells expressing PD-1. The culture of naive TCD4 cells with naive B cells alone did not produce Tfh cells. However, when dendritic cells were added, Tfh cells expressing PD-1 increased significantly.³⁸ Hemin is responsible for activating HO-1, which is responsible for inducing dendritic cell responses.³⁹

Tfh cells expressing PD-1 increased in both groups after hemin administration in this study, possibly due to hemin inducing HO-1. This process activates dendritic cell responses, increasing Tfh cells expressing PD-1. The increase in Tfh cells expressing PD-1 inhibits Tfh cell activation against specific antigen stimulation, making Tfh cells unable to induce B cells to form antibodies. This inability might have a protective effect against transfusion-dependent thalassemia after repeated exposure to red cell antigens that are different from the self-antigens.

Our study showed that Tfh cells expressing IL-21 did not differ significantly between groups before and after hemin administration. Tfh cells produce IL-21 in response to antigens presented by dendritic cells, inducing B cell differentiation in germinal centers. One study reported that circulating Tfh cells also had memory capabilities.⁴⁰ Tfh cells expressing IL-21 did not differ between groups, possibly due to repeated transfusions resulting in exposure to different red cell antigens under the same conditions in both groups that were not affected by hemin.

The results showed a higher median percentage of Tfh cells expressing IL-21 in both groups but a wide interval compared to osteoarthritis patients in another study.⁴¹ Studies on lymphocyte cells from mouse lymph nodes have shown that IL-21 mediates the growth and survival of Tfh cells. The IL-21 receptor is expressed on Tfh cells and B cells, suggesting a central role of IL-21 in Tfh cell formation and antibody production. Tfh cells are known to express ICOS and IL-21. ICOS expression has an impact on IL-21 production.⁴²

Another study that cocultured circulating Tfh cells with dendritic cells showed that dendritic cells led to increased Tfh cell activation and cytokine production, including IL-21, but IL-21 also inhibited dendritic cell activation and maturation.⁴³ Studies in patients with Sjogren's syndrome with positive antibodies have reported circulating Tfh cells expressing higher IL-21 and positively correlated with IgG levels.⁴⁴ We suspect that hemin administration did not increase Tfh cells expressing IL-21 because hemin is not a specific antigen presented by dendritic cells to Tfh cells, leading to a weaker effect on increasing IL-21 expression. In addition, the number of Tfh cells expressing IL-21 was already high, which might be sufficient to maintain Tfh cell activation and survival.

We recognize that this study has limitations, so it cannot comprehensively describe the immune response of the two thalassemia groups. Some factors that affected the study results cannot be completely ruled out. The level of heme in the blood was not measured, so the hemolysis level of individual subjects was not evaluated. This study did not measure the transcription factor nuclear transcriptional repressor B cell lymphoma 6 (BCL6), the CXCR5, an inducible co-stimulator (ICOS) that affects the immune response of T cells, including Tfh, to stimulate B cells forming antibodies. This study was limited to the Tfh immune response, while the hemolysis-related immune response involved the innate immune response. We also did not examine T regulators, which control T effector, including Tfh cells.

Conclusions

Tfh cells did not differ significantly between thalassemia groups before hemin administration, but a significant increase was observed after hemin administration. The increase in Tfh cells was higher in allo-auto positive thalassemia patients. Tfh cells expressing TIGIT did not differ significantly between thalassemia groups, but a significant decrease was observed after hemin administration. Tfh cells expressing PD-1 did not differ significantly between thalassemia groups, but hemin administration increased Tfh cells expressing PD-1 in both groups. Tfh cells expressing IL-21 did not differ significantly between thalassemia groups before and after hemin administration. The immune response of Tfh cells expression did not differ between allo-auto positive and negative thalassemia. Hemin administration affected Tfh cell expression in both thalassemia groups.

Data Sharing Statement

The statistical data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The study was approved by the Health Research Ethics Committee of the Dr. Soetomo General Academic Hospital (no. 0527/LOE/301.4.2/VII/2021).

Acknowledgments

We acknowledge all participants for supporting and cooperating in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this article.

References

1. Viprakasit V, Ekwattanakit S. Clinical classification, screening and diagnosis for thalassemia. *Hematol Oncol Clin North Am*. 2018;32:193–211. doi:10.1016/j.hoc.2017.11.006
2. D'Arqom A. Nucleic acid therapy for β -thalassemia. *Biologics*. 2020;14:95–105.
3. Jazuli MI, Bintoro SUY, Mudjanarko SW. The association between serum ferritin levels and 25(OH)D levels in adult patients with transfusion dependent thalassemia. *J Med Chem Sci*. 2022;5:35–41.
4. Fonda T, Novida H. Management of gestational diabetes mellitus in a beta major thalassemia patient. *Curr Intern Med Res Pract Surabaya J*. 2021;2:45. doi:10.20473/cimrj.v2i2.26255
5. Suryawan A, Ningtiar HW, Irwanto I, et al. Determinant factors of depression in beta major thalassemia children. *Folia Medica Indones*. 2021;57:46. doi:10.20473/fmi.v57i1.13664
6. Meulenbroek EM, Wouters D, Zeerleder SS. Lyse or not to lyse: clinical significance of red blood cell autoantibodies. *Blood Rev*. 2015;29:369–376. doi:10.1016/j.blre.2015.05.001
7. Tangvarasittichai S. Impact of alloimmunization on transfusion-dependent patient. *Ann Adv Chem*. 2017;1:70–82. doi:10.29328/journal.aac.1001009
8. Ben KM, Ouederni M, Sahli N, et al. Predictors of autoimmune hemolytic anemia in beta-thalassemia patients with underlying red blood cells autoantibodies. *Blood Cells, Mol Dis*. 2019;79. doi:10.1016/j.bcmd.2019.102342
9. Al-Riyami AZ, Al-Muqbali A, Al-Sudiri S, et al. Risks of red blood cell alloimmunization in transfusion-dependent β -thalassemia in Oman: a 25-year experience of a university tertiary care reference center and a literature review. *Transfusion*. 2018;58:871–878. doi:10.1111/trf.14508
10. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. *Transfusion*. 2012;52:2220–2224. doi:10.1111/j.1537-2995.2012.03570.x

11. Seferi I, Xhetani M, Face M, et al. Frequency and specificity of red cell antibodies in thalassemia patients in Albania. *Int J Lab Hematol*. 2015;37:569–574. doi:10.1111/ijlh.12362
12. Vichinsky E, Neumayr L, Trimble S, et al. Transfusion complications in thalassemia patients: a report from the CDC. *Transfusion*. 2015;54:1–20.
13. Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev*. 2006;86:583–650. doi:10.1152/physrev.00011.2005
14. Zhong H, Bao W, Friedman D, et al. Hemin controls T cell polarization in sickle cell alloimmunization. *J Immunol*. 2014;193:102–110. doi:10.4049/jimmunol.1400105
15. Martins R, Knapp S. Heme and hemolysis in innate immunity: adding insult to injury. *Curr Opin Immunol*. 2018;50:14–20. doi:10.1016/j.coi.2017.10.005
16. Vingert B, Tamagne M, Habibi A, et al. Phenotypic differences of CD4+ T cells in response to red blood cell immunization in transfused sickle cell disease patients. *Eur J Immunol*. 2015;45:1868–1879. doi:10.1002/eji.201445187
17. Godefroy E, Zhong H, Pham P, et al. TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: potential role in sickle cell alloimmunization. *Haematologica*. 2015;100:1415–1425. doi:10.3324/haematol.2015.132738
18. Saifelddeen E, Awad M, El-Tonbary Y, et al. Risk for red cell immunization among thalassemic patients. *Egypt J Haematol*. 2017;42:58. doi:10.4103/1110-1067.216113
19. Datta SS, Mukherjee S, Talukder B, et al. Frequency of red cell alloimmunization and autoimmunization in thalassemia patients: a report from Eastern India. *Adv Hematol*. 2015;2015:1–6. doi:10.1155/2015/610931
20. Raul Badell I, Ford ML. Follicular Helper T. Cells in the generation of alloantibody and graft rejection. *Physiol Behav*. 2016;176:139–148.
21. Gao Y, Jin H, Nan D, et al. The Role of T follicular helper cells and T follicular regulatory cells in the pathogenesis of autoimmune hemolytic anemia. *Sci Rep*. 2019;9:1–11. doi:10.1038/s41598-019-56365-3
22. Brenna E, Davydov AN, Ladell K, et al. CD4+ T follicular helper cells in human tonsils and blood are clonally convergent but divergent from non-Tfh CD4+ cells. *Cell Rep*. 2020;30:137–152.e5. doi:10.1016/j.celrep.2019.12.016
23. Crotty S. T follicular helper cell differentiation, function and roles in disease. *Immunity*. 2015;41:529–542.
24. Laguna-Goya R, Suárez-Fernández P, Paz-Artal E. Follicular helper T cells and humoral response in organ transplantation. *Transplant Rev*. 2019;33:183–190. doi:10.1016/j.trre.2019.06.003
25. Schey KL, Luther JM, Rose KL. Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol*. 2016;16:1–21.
26. Paufl SM, Miller SC. Follicular helper T cells: lineage and location. *Bone*. 2012;78:711–716.
27. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. *J Clin Invest*. 2017;127:750–760. doi:10.1172/JCI89741
28. Zhong H, Yazdanbakhsh K. Hemolysis and immune regulation. *Curr Opin Hematol*. 2018;25:177–182. doi:10.1097/MOH.0000000000000423
29. Luo Q, Ye J, Zeng L, et al. Elevated expression of TIGIT on CD3+CD4+ T cells correlates with disease activity in systemic lupus erythematosus. *Allergy Asthma Clin Immunol*. 2017;13:1–12. doi:10.1186/s13223-017-0188-7
30. Kurtulus S, Sakuishi K, Ngiow SF, et al. TIGIT predominantly regulates the immune response via regulatory T cells. *J Clin Invest*. 2015;125:4053–4062. doi:10.1172/JCI81187
31. Ma JL, Yang PY, Rui YC, et al. Hemin modulates cytokine expressions in macrophage-derived foam cells via heme oxygenase-1 induction. *J Pharmacol Sci*. 2007;103:261–266. doi:10.1254/jphs.FP0060270
32. Chauveau C, Rémy S, Royer PJ, et al. Heme oxygenase-1 expression inhibits dendritic cell maturation and proinflammatory function but conserves IL-10 expression. *Blood*. 2005;106:1694–1702. doi:10.1182/blood-2005-02-0494
33. Byford ET, Carr M, Ladikou E, et al. Circulating Tfh1 (cTfh1) cell numbers and PD1 expression are elevated in low-grade B-cell non-Hodgkin's lymphoma and cTfh gene expression is perturbed in marginal zone lymphoma. *PLoS One*. 2018;13:1–18. doi:10.1371/journal.pone.0190468
34. Bardhan K, Anagnostou T, Boussiotis VA. The PD1: PD-L1/2 pathway from discovery to clinical implementation. *Front Immunol*. 2016;7. doi:10.3389/fimmu.2016.00550
35. Shi J, Hou S, Fang Q, et al. PD-1 controls follicular T helper cell positioning and function. *Immunity*. 2018;49:264–274.e4. doi:10.1016/j.immuni.2018.06.012
36. Jogdand GM, Mohanty S, Devadas S. Regulators of Tfh cell differentiation. *Front Immunol*. 2016;7:1–14. doi:10.3389/fimmu.2016.00520
37. Sage PT, Francisco LM, Carman CV, et al. PD-1 controls lymph node and blood T follicular regulatory cells. *Nat Immunol*. 2013;14:152–161. doi:10.1038/ni.2496
38. Martin-Gayo E, Cronin J, Hickman T, et al. Circulating CXCR5+CXCR3+PD-1^{hi} Tfh-like cells in HIV-1 controllers with neutralizing antibody breadth. *JCI Insight*. 2017;2. doi:10.1172/jci.insight.89574
39. Thomas RAB, Czopek A, Bellamy COC, et al. Hemin preconditioning upregulates heme oxygenase-1 in deceased donor renal transplant recipients: a randomized, controlled, phase IIB Trial. *Transplantation*. 2016;100:176–183. doi:10.1097/TP.0000000000000770
40. Sage PT, Alvarez D, Godec J, et al. Circulating T follicular regulatory and helper cells have memory-like properties. *J Clin Invest*. 2014;124:5191–5204. doi:10.1172/JCI76861
41. Shan Y, Qi C, Liu Y, et al. Increased frequency of peripheral blood follicular helper T cells and elevated serum IL-21 levels in patients with knee osteoarthritis. *Mol Med Rep*. 2017;15:1095–1102. doi:10.3892/mmr.2017.6132
42. Vogelzang A, McGuire HM, Yu D, et al. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity*. 2008;29:127–137. doi:10.1016/j.immuni.2008.06.001
43. Patente TA, Pinho MP, Oliveira AA, et al. Human dendritic cells: their heterogeneity and clinical application potential in cancer immunotherapy. *Front Immunol*. 2019;10:1–18. doi:10.3389/fimmu.2019.00001
44. Pontarini E, Murray-Brown WJ, Croia C, et al. Unique expansion of IL-21+ Tfh and Tph cells under control of ICOS identifies Sjögren's syndrome with ectopic germinal centres and MALT lymphoma. *Ann Rheum Dis*. 2020;79:1588–1599. doi:10.1136/annrheumdis-2020-217646

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Prevalence of ABO and Rh Blood Group Among Volunteer Blood Donors at the Blood and Tissue Bank Service in Addis Ababa, Ethiopia

Getu Jenbere Debele, Fekadu Urgessa Fita & Melatwork Tibebe

To cite this article: Getu Jenbere Debele, Fekadu Urgessa Fita & Melatwork Tibebe (2023) Prevalence of ABO and Rh Blood Group Among Volunteer Blood Donors at the Blood and Tissue Bank Service in Addis Ababa, Ethiopia, Journal of Blood Medicine, , 19-24, DOI: [10.2147/JBM.S392211](https://doi.org/10.2147/JBM.S392211)

To link to this article: <https://doi.org/10.2147/JBM.S392211>



© 2023 Debele et al.



Published online: 18 Jan 2023.



Submit your article to this journal



Article views: 807



View related articles



View Crossmark data



Citing articles: 8 View citing articles

Prevalence of ABO and Rh Blood Group Among Volunteer Blood Donors at the Blood and Tissue Bank Service in Addis Ababa, Ethiopia

Getu Jenbere Debele¹, Fekadu Urgessa Fita², Melatwork Tibebe²

¹Ethiopian Blood and Tissue Bank Service, Addis Ababa, Ethiopia; ²College of Health Science, Department of Medical Laboratory Science, Addis Ababa University, Addis Ababa, Ethiopia

Correspondence: Melatwork Tibebe, Email Melatwork.tibebe@aau.edu.et

Background: The discovery of the ABO blood group system and testing of blood donors highly reduced the fatalities associated with blood transfusion reactions and improved the safety of blood transfusion. Blood group antigens are found on the surface of red blood cells that are inherited biological characteristics that do not change throughout life in healthy individuals.

Objective: To determine the prevalence ABO and Rh blood groups Among Volunteer Blood Donors at Ethiopian blood and tissue bank service (EBTBS), Addis Ababa.

Methods: A cross-sectional study was carried out from January 2022 to May 2022, on 1700 volunteer blood donors to assess prevalence of ABO and Rh blood groups among volunteer blood donors at the Ethiopian blood and tissue bank service. All tests were performed using fully automated immunohematology analyzer (Galileo Neo Immucor). Data processing and analysis were undertaken by using Statistical Package for the Social Sciences (SPSS) version 26. An ethical clearance letter was obtained from Addis Ababa University and informed consent was also obtained from the participants of the study.

Results: A total of 1700 donors were included, of which 57% of donors were males. The majority of the donors belonged to the age group between 18 and 25 years old (53%). The antigen frequencies of ABO and Rh(D) blood group system showed that O was the most prevalent blood group 44.65% followed by A (28.41%), B (21.24%), and AB (5.71%). The Rh-positive donors were more prevalent (94.82%) as oppose to the Rh-negative (5.18%).

Conclusion: The knowledge of the distribution of blood groups is very important for blood banks and transfusion services which play an important role in the patient's health care. The findings of the ABO blood group in this study were comparable to other studies conducted in Ethiopia.

Keywords: ABO blood group, Rh factor

Introduction

The discovery of the ABO blood group system and testing of blood donors highly reduced the fatalities associated with blood transfusion reactions and improved the safety of blood transfusion.¹ Blood group antigens are inherited biological characteristics that do not change throughout life in healthy people and represent antigens found on the surface of red blood cells (RBCs).² The International Society of Blood Transfusion (ISBT) currently recognized 43 blood group systems containing 345 red cell antigens (June 2021). The ISBT also maintains three categories of antigens that have not yet been associated with blood group systems. "Collections" (the 200 series) were designed to group antigens that are biochemically, genetically, or serologically similar where the genetic basis has not yet been discovered. The "700 Series" contains antigens that do not fit into any system or collection and have an incidence of less than 1% across all human ethnic populations, and the "901 Series" contains antigens that have a frequency of greater than 99% across populations of different ethnic ancestry.³

The ISBT acknowledged 9 blood group systems that are clinically significant and associated with hemolytic transfusion reactions (HTR), and hemolytic disease in fetuses and newborns (HDFN).⁴ These blood group systems are

ABO, Rhesus, Kell, Duffy, Kidd, MNS, P, Lewis, and Lutheran. Out of these blood group systems, antigens of the three blood group systems are important in clinical transfusion practices, which are the ABO, Rh, and Kell blood group systems are highly immunogenic⁵ The ABO and Rhesus (Rh) blood group system is most important for blood transfusion purposes, parental testing, legal medicine, and in population genetic study.⁶

WHO blood safety initiative described that the quality and safety of blood and blood products must be assured throughout the process from the selection of blood donors to the administration of blood to the patient.⁷ Safe blood transfusion is not realized only by testing of blood for transfusion transmissible Infections (TTIS), but also thorough protection from HTR resulting from Alloimmunization against red cell antigens,⁸ which commonly occurs following a transfusion or in pregnancy.⁹ The primary responsibility of all blood bank services was to ensure safe blood and blood products for the transfusing facilities and recipients for routine use. Laboratory testing of the donor's blood is important to ensure that the recipient receives the safest possible blood. Transfusing phenotype-matched blood can prevent the development of antibodies against clinically significant antigens. However, the problem is greater in developing countries, especially in resource-limited countries like Ethiopia, as the lack of awareness, and typing antisera as well as the associated costs and logistics were a serious concern. The primary outcome of this study is to detect the prevalence of ABO and Rh-D blood groups among voluntary blood donors at EBTBS, Addis Ababa.

The results of this study will be beneficial for health personnel, planners, policymakers, Non-Governmental Organizations, and others who are engaged in Blood transfusion activities. Hence, the findings of this study will be disseminated to the relevant bodies, stakeholders, and others who are involved in improving the safety of blood donation. This study can also be used to establish guidelines for blood group distribution throughout the country, used as a baseline contribution for the researchers, and additional input for EBTBS and MoH.

Methods

This study was carried out between January and May, 2022 at the Ethiopian Blood and Tissue Bank (EBTBS), located in Addis Ababa, the capital city of Ethiopia. The blood bank service in Ethiopia was established in 1969 by the Ethiopian Red Cross Society (ERCS). The Government made a policy decision to revert the responsibility from ERCS to a Government-owned service under the Federal Ministry of Health (FMoH) as an autonomous agency as of January 8, 2015. Currently, since January 25, 2022, the blood bank has been re-established as an EBTBS under Regulation Number No. 1263/2021, tasked to coordinate, give logistic and technical support as well as oversee all the 43 regional blood bank services by managing and controlling tissue and organ transplantation in the country.

A cross-sectional study was carried out to determine the frequency of ABO and Rh blood groups in volunteer blood donors. Blood donors who were available during the data collection period were selected after proper history taking and complete examination for fulfilling the eligibility criteria to donate blood. A convenient sampling method was used for sample collection. All blood samples that were collected at EBTBS during the study period were the source population and those blood samples that were used for the study during the study period were the study population.

The sample size was calculated using the single population proportion formula with a proportion of 50%. The 2.5% margin of error was used to increase accuracy. A 95% of a Confidence interval and 10% for an inconvenient sample were used to determine the required sample size for the study.

Where: - n: Estimated sample size.
$$n = \frac{\left(Z_{\alpha}\right)^2 P(1-P)}{d^2}$$

Z: Z statistic for a level of confidence, Z=1.96 (from normal distribution table for CI of 95%)

p: Expected prevalence or proportion, 50%, because there is no previous study in Addis Ababa (in a proportion of one; if 50%, $P = 0.5$), d: precision 2.5%, which is a tolerable error between the sample and the true population. (In proportion of one; if 2.5%, $d = 0.025$). Then $n = \frac{(1.96)^2 * 0.50 * (0.50)}{(0.025)^2} = \frac{0.9604}{0.000625} = 1537$

Considering a 10% inconvenient sample ($1537 \times 10\% = 153$) The estimated sample size was $1537 + 153 = 1690$. However, additional 10 samples were added to make a sample size of 1700.

A total of 1700 blood samples, including 970 (57.1%) males and 730 (42.9%) females from volunteer healthy blood donors between the ages of 18 and 65, weighing more than 45 kilograms with normal hemoglobin values were included

in the study. The Blood donors were selected strictly according to the nationally standardized selection criteria established by the blood bank service. A questionnaire was given to each donor, that includes details regarding their basic Socio-demographic profile, age, sex, address, any history of the previous transfusion, drug intake, jaundice, and any clinically significant disease related to autoimmune disorders. The general health conditions of blood donors were assessed through a medical examination.

Laboratory Procedure

Blood specimens were collected using a standard phlebotomy technique, by well-trained nurses, from volunteer non-remunerated blood donors, through a mobile campaign, and at the center clinic. A 5 mL of blood sample was collected at the time of donation into a tube containing ethylenediaminetetraacetic acid (EDTA). From the mobile collection site, blood specimens were transported to the blood bank by maintaining the cold chain at 2–10°C with a cold box. After arrival at the blood bank, the blood samples were centrifuged at 3000 rpm for 5 min.

Blood samples were analyzed to determine ABO and Rh-D blood groups; using the Galileo fully automated immunohematology analyzer (Galileo Neo Immucor Gamma automated system). The ABO forward and, reverse grouping, as well as Rh-D tests, were performed based on the agglutination principle with microplates. Forward grouping was done using anti-A, anti-B, anti-AB, and anti-D antisera. Reverse grouping was done using A1 and B red blood cells. Donors typed as Rh-D negative were confirmed using an antiglobulin weak D test in an automated solid-phase test using Novaclone anti-D (Immunocor Med. Diagnostik GmbH, Germany) IgG and IgM antisera. The sample was considered Rh (D) negative only after the “weak D” testing was confirmed to be negative.

Quality Assurance

Blood samples were collected by well-trained nurses, after obtaining written or oral informed consent from the volunteer blood donors. Universal precautions were taken during sample handling, processing, and testing. Reagent storage and labeling of samples were managed properly according to the kit manufacturer (Immucor, Inc, Germany) and EBTBS standards. All data recordings were checked for completeness as well as the results were recorded with the donor identification number.

Daily quality control of selected red blood cells and antiserum was performed to confirm the reactivity and specificity of the reagent. These reagents were tested with the corresponding antigen-positive and antigen-negative red blood cells. The reagents were considered appropriate for use if only antigen-positive red blood cells demonstrate a positive result. To establish the validity of the results’ of the automated system, the initial 30 samples were typed manually using the tube method in parallel to the study using reagents from Immucor Inc. The manual testing was done according to the manufacturers’ (Immucor, Inc, Germany) instructions. No discrepancies were found in any of these tests.

The collected data was cleaned, coded, summarized, entered into an Excel spreadsheet, and imported to the statistical package for social science version 26 (SPSS Inc. Chicago, IL, USA) for analysis and interpretation. The Data were analyzed using descriptive statistical analysis utilizing absolute frequencies and percentages, and the results were organized in [Tables 1](#) and [2](#). *P*-value of <0.05 was considered statistically significant with a 95% confidence level.

The research proposal was ethically cleared by the Department Research and Ethical Review Committee at the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Official permission from EBTBS was obtained as well. We confirm that our study complies with the Declaration of Helsinki. During data collection, all blood donors gave their consent that their blood sample can be used for research purposes and no donor name was recorded. Blood samples were labeled only with the donor identification number used as a unique study code. Confidentiality of the result was maintained, and the results were shared only with the medical staff of authorized members of the study. Computerized files were password protected and paper files were also locked safely and accessible only to authorized personnel.

Result

A total of 1700 volunteer donor samples were analyzed to determine the prevalence blood groups. Male predominance in blood donation accounted for 57.1% (970/1700). The younger age group (18–25 years) contributed about 53.1% (903/

Table 1 Blood Group and Rh(D) Prevalence Among Voluntary Blood Donors and the Correlation with Gender from January 2022 to May 2022 at EBTBS (n=1700)

ABO	Rh(D) Positive		Rh(D) Negative		Total	P value
	Male	Female	Male	Female		
A	267 (15.71%)	199 (11.71%)	11 (0.65%)	5 (0.29%)	482 (28.35%)	0.3
B	182 (10.71%)	160 (9.41%)	7 (0.41%)	13 (0.76%)	362 (21.29%)	
AB	53 (3.12%)	40 (2.35%)	1 (0.06%)	3 (0.18%)	97 (5.71%)	
O	421 (24.76%)	291 (17.12%)	28 (1.65%)	19 (1.12%)	759 (44.65%)	
Total	923 (54.29%)	690 (40.59%)	47 (2.76%)	40 (2.35%)	1700 (100%)	

Table 2 ABO and Rh (D) Blood Group Distribution in Ethiopian Blood Donors

ABO & Rh (D) Blood Group	Current Study		Jimma	Bahirdar	Gondar	Arbaminch	Jiggjiga	Debre Tabor
A	483	28.4%	31.9%	29.8%	26.4%	32.7%	29.6%	29.8%
B	361	21.3%	21.5%	23.2%	21.7%	20.9%	15.2%	23.2%
AB	97	5.7%	3.5%	5.5%	4.9%	4.3%	5.1%	5.5%
O	759	44.6%	43.1%	41.5%	47.0%	42.1%	50.2%	41.5%
Rh (D) Positive	1612	94.8%	92.8%	91.5%	94.2%	92.8%	95.6%	91.5%
Rh (D) Negative	88	5.2%	7.2%	8.5%	5.8%	7.2%	4.4%	8.5%

1700) of the total blood donations followed by 26–35, 36–45, 46–55, and 56–65 years of age groups accounting for 25.9% (441/1700), 14.3% (243/1700), 5.2% (88/1700) and 1.5% (25/1700) respectively. The mean age of the donors was 28 years, the median age was 24 years and the most frequently donating age group was among 19-year-olds.

The distribution of ABO and Rh blood groups showed that, O was the most prevalent blood group 559(44.65%) followed by A 483(28.41%), B 361(21.24%), and AB 97(5.71%) of the total volunteer donors in the study. The distributions of O positive blood group were the highest (41.88%), followed by A positive (27.41%), B positive (20.12%), AB positive (5.47%), O negative (2.76%), B negative (1.18%), A negative (0.94%), and AB negative (0.24%). Rh-positive donors were more prevalent (94.82%) as opposed to Rh-negative (5.18%). ABO and Rh frequency were not significantly different between male and female donors ($P = 0.3$) (Table 1). There was also no statistically significant correlation between age and blood group ($P = 0.2$).

The most dominant blood group was found to be O (44.5%) and the least common AB (5.7%). The Rh-positive blood group was also the most predominant which covered 94.8% and the rest (5.2%) were Rh-negative (Table 2).

Discussion

To the best of our knowledge, our study is the first study to be done in Addis Ababa, not to mention the larger sample size and the use of fully automated immunohematology machine. In our study, we found the majority of volunteer donors to be male donors. The studies conducted in other countries also show a predominance of male donors; for instance; a study done by El-Faramawy et al reported that 65% of the donors were male and only 35% were female.¹⁷ Another study by Luhar et al reported in their study that 95.27% were male.¹⁸ The study done in South India, by Sachan et al,¹⁹ also found 96% of donors to be male. In our study, however, the percentage of female donors is relatively high in comparison to other studies, which may be because females have better awareness on blood donation service at the EBTBS, Addis Ababa center.

This study was comparable with other studies in Ethiopia and most regions of Africa.^{10–16} However, very good improvement was seen in volunteer turnouts and overall awareness about the importance of blood donation. The predominance of male donors is observed might be because female donors are more likely to be excluded due to medical backgrounds, such as low hemoglobin levels, low body weight, pregnancy, or breast feeding. The younger age group (18–25 years) were the most common blood donors, contributing about 53.1% of the total blood donations, this might be due to the younger population may have better awareness, good physical health, easy understanding, and be easily convinced group.

In this study, the most predominant blood group was O (44.5%) and the least common AB (5.7%) which is consistent with studies in other regions of Ethiopia (Table 2) which also showed the predominant group to be O and the least common to be AB.^{20–23} In Kenya, Tanzania, and Uganda, similar studies also showed the predominant blood group to be O and the least prevalent to be AB.^{12–14} On contrary, some studies in India showed that it was blood group B which was the dominant one,^{24,25} while studies in Iran and Europe indicated that blood group A was the predominant blood group.²⁶ The Rh-positive blood group was also the most predominant which covered 94.8%, similarly, a study conducted in Jimma, Bahir Dar, Gondar, Arba Minch, Jigjiga, and Debre Tabor regions of Ethiopia, indicated that Rh positive blood group was the most prevalent with 92.8%, 91.5%, 94.2%, 92.8%, and 95.6% and 91.5% frequency respectively.^{10,11,20–23} Although, a study conducted in Gambela region of Ethiopia reported a slightly higher frequency of 19.37% for the Rh-negative blood group.²⁷ Consistent with the other studies, a study conducted in Tanzania, and Uganda showed that Rh D negative frequency to be 2% for both countries.^{13,14}

This difference in the distribution of the ABO blood group might be due to geographic, regional, and ethnic differences as well as genetic variations in the study groups.

Limitation

The study was conducted on volunteer blood donors who came to the center located downtown Addis Ababa and some mobile sites which may have inadvertently led to conducting the study on somewhat a homogenous population.

Conclusion

The knowledge of the distribution of blood groups is very important for blood banks and transfusion services which play an important role in the patient's health care. We recommend a larger nation-based study involving blood donors from different regions and backgrounds in Ethiopia to confirm these findings and to assess for any region-specific variations in the ABO blood group and Rh-D antigens among blood donors considering the heterogeneous geography, ethnicity, and genetic variation of the country.

Acknowledgments

First of all, our great respect and gratitude goes to volunteer blood donors, nurses, and laboratory technologists of the EBTBS, who had taken full responsibility for interviewing participants and collecting blood samples despite having the usual heavy workload. Last but not least, our deepest gratitude also goes to EBTBS for covering the cost of reagents and giving us chance to use laboratory facilities to perform the test analysis.

Disclosure

The authors report no conflicts of interest regarding the publication of this paper.

References

1. Garratty G, van Rhenen DJ. Red blood cell alloimmunization after blood transfusion. *Leiden Univ Press*. 2008;870:11–12.
2. Milanović MK, Bujandrić N, Knezević NM. PROBLEM DAVALACA KRV I RETKI H KRVNIH GRUPA SA IREGULARNIM ANTITELIMA [Rare blood donors with irregular antibodies]. *Med Pregl*. 2013;66(7–8):331–334. Serbian. doi:10.2298/MPNS1308331K
3. Smart E, Armstrong B. Blood group systems. *ISBT Sci Series*. 2020;15(S1):123–150. doi:10.1111/voxs.12593
4. Tobian AAR, Heddle NM, Wiegmann TL, Carson JL. Red blood cell transfusion: 2016 clinical practice guidelines from AABB. *Transfusion*. 2016;56(10):2627–2630. doi:10.1111/trf.13735

5. Sharma S, Sharma DC, Rai S, et al. Prevalence of ABO, RhD and other clinically significant Blood Group Antigens among blood donors at tertiary care center, Gwalior. *Bali Med J*. 2020;9(2):437. doi:10.15562/bmj.v9i2.1779
6. Singh A, Srivastava R, Deogharia K, Singh K. Distribution of ABO and Rh types in voluntary Blood donors in Jharkhand area as a study conducted by RIMS, Ranchi. *J Family Med Prim Care*. 2016;5(3):631. doi:10.4103/2249-4863.197319
7. Chaurasia R, Zaman S, Das B, Chatterjee K. Screening donated blood for transfusion transmitted infections by serology along with NAT and response rate to notification of reactive results: an Indian experience. *J Blood Transfus*. 2014;2014:1–6. doi:10.1155/2014/412105
8. Rath G, Mitra R, Mishra N. Blood groups systems. *Indian J Anaesth*. 2019;58(5):524–528. doi:10.4103/0019-5049.144645
9. Chauhan DL, Premkumar DR. Significance of antibody screening and identification in pretransfusion testing-a retrospective study. *Semantic Scholar*. 2017;6(5):2319–7064.
10. Alemu G, Mama M. Assessing ABO/Rh Blood Group frequency and association with asymptomatic malaria among blood donors attending Arba Minch Blood Bank, South Ethiopia. *Malar Res Treat*. 2016;2016(1):1–7. doi:10.1155/2016/8043768
11. Sheikaden A. Somali Regional Blood Bank. *Am J Lab Med*. 2019;4(2):48–52.
12. Lyko J, Gaertner H, Kaviti JN, Kariithi MW, Akoto B. Grupy krwi układu ABO i Rh u ludności Kenii [Blood-group systems ABO and RH in the Kenyan population]. *Folia Med Cracov*. 1992;33(1–4):85–92. Polish.
13. Apecu RO, Mulogo EM, Bagenda F, Byamungu A. ABO, and Rhesus (D) blood group distribution among blood donors in rural south-western Uganda: a retrospective study. *BMC Res Notes*. 2016;9(1). doi:10.1186/s13104-016-2299-5
14. Jahanpour O, Pyuza JJ, Ntiyakunze EO, Mremi A, Shao ER. ABO and Rhesus blood group distribution and frequency among blood donors at Kilimanjaro Christian Medical Center, Moshi, Tanzania. *BMC Res Notes*. 2017;10(1). doi:10.1186/s13104-017-3037-3
15. Enawgaw B, Aynalem M, Melku M. Distribution of ABO and Rh-D blood group antigens among blood donors in the Amhara Regional State, Ethiopia. *J Blood Med*. 2022;13(1):97–104. doi:10.2147/JBM.S356425
16. Amsalu WF, Daniel GD. Distribution of ABO and Rh (D) blood groups among students attending secondary and preparatory schools in Bote town, Oromia national regional state, Ethiopia. *Int J Sci Technol Educ Res*. 2019;10(1):1–8. doi:10.5897/IJSTER2019.0448
17. Jungbauer C, Hobel CM, Schwartz DWM, Mayr WR. High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors. *Vox Sang*. 2012;102(3):234–242. doi:10.1111/j.1423-0410.2011.01542.x
18. Luhar RK, Shah RJ, Harimoorthy V. Antibody screening and identification in voluntary blood donors – need of the hour. *Global J Transfus Med*. 2022;7(1):47. doi:10.4103/gjtm.gjtm_1_22
19. Sachan D, Krishna GD, Saha S, Prasath R. Prevalence of irregular red blood cell antibodies among healthy blood donors in South India. *Glob J Transfus Med*. 2019;4:219–223. doi:10.4103/GJTM.GJTM_22_19
20. Zerihun T, Bekele S. Pattern of ABO and Rhesus Blood Groups distribution of five years survey in Jimma Town Blood Bank, South West Ethiopia. *J Health Educ Res Dev*. 2016;4(3). doi:10.4172/2380-5439.1000177
21. Legese B, Shiferaw M, Tamir W, Tiruneh T. Distribution of ABO and Rhesus Blood Group phenotypes among blood donors at Bahir Dar Blood Bank, Amhara, Northwest Ethiopia: a retrospective cross-sectional study. *J Blood Med*. 2021;12(1):849–854. doi:10.2147/JBM.S329360
22. Woldu B, Melku M, Shiferaw E, Biadgo B, Abebe M, Gelaw Y. Phenotype, allele, and genotype frequency of ABO and Rhesus D blood groups of blood donors at the North Gondar District Blood Bank, Northwest Ethiopia. *J Blood Med*. 2022;13(1):11–19. doi:10.2147/JBM.S346904
23. Tiruneh A, Yetneberk T, Gelaw M, Eshetie D. Frequency of ABO and Rh blood group distribution at Debre Tabor blood bank, Amhara Region, North-Central Ethiopia. A six-year retrospective survey. *J Blood Med*. 2020;11(1):357–361. doi:10.2147/JBM.S266624
24. Chandra T, Gupta A. Frequency of ABO and rhesus blood groups in blood donors. *Asian J Transfus Sci*. 2012;6(1):52. doi:10.4103/0973-6247.95057
25. Barot T, Patel D, Shah R. Distribution of ABO and Rhesus (Rh) blood groups among voluntary blood donors in Central Gujarat, India. *Int J Contemp Med Res*. 2020;7(7):114.
26. Babaei K, Esmaeilzadeh A, Asadi S, Sohrabi R. Prevalence of red blood cell alloantibodies in blood donors of Zanjan Province; the preliminary report of the North West of Iran. *Biosci Biotechnol Res Asia*. 2016;13(4):2207–2210. doi:10.13005/bbra/2385
27. Golassa L, Tsegaye A, Erko B, Mamo H. Correction to: high rhesus (Rh(D)) negative frequency and ethnic-group based ABO blood group distribution in Ethiopia. *BMC Res Notes*. 2018;11(1). doi:10.1186/s13104-018-3281-1

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Platelet Parameters and Their Correlation with Parasitemia Levels Among Malaria Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia: Comparative Cross-Sectional Study

Haftu Asmerom, Kabtamu Gemechu, Tilahun Bete, Beza Sileshi, Berhe Gebremichael, Muluken walle & Mesay Arkew

To cite this article: Haftu Asmerom, Kabtamu Gemechu, Tilahun Bete, Beza Sileshi, Berhe Gebremichael, Muluken walle & Mesay Arkew (2023) Platelet Parameters and Their Correlation with Parasitemia Levels Among Malaria Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia: Comparative Cross-Sectional Study, Journal of Blood Medicine, , 25-36, DOI: [10.2147/JBM.S394704](https://doi.org/10.2147/JBM.S394704)

To link to this article: <https://doi.org/10.2147/JBM.S394704>



© 2023 Asmerom et al.



Published online: 19 Jan 2023.



Submit your article to this journal



Article views: 556



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Platelet Parameters and Their Correlation with Parasitemia Levels Among Malaria Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia: Comparative Cross-Sectional Study

Haftu Asmerom¹, Kabtamu Gemechu¹, Tilahun Bete², Beza Sileshi¹, Berhe Gebremichael³, Muluken walle⁴, Mesay Arkew¹

¹School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ²School of Nursing and Midwifery, Department of Psychiatry, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ³School of Public Health, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ⁴Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Jigjiga University, Jigjiga, Ethiopia

Correspondence: Haftu Asmerom, School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, P.O. Box 235, Harar, Ethiopia, Tel +251913889039, Email wawhaftu12@gmail.com

Background: Malaria is a major public health problem with the highest morbidity and mortality in developing countries. Hematological changes play a great role in malaria pathogenesis through platelets and platelet parameters. However, the changes in platelet parameters are not clearly described in Ethiopia. Therefore, this study aimed to compare platelet parameters and their correlation with parasitemia among malaria-infected adult patients and healthy adults.

Methods: An institutional-based comparative cross-sectional study was conducted involving 186 (93 malaria-infected patients and 93 healthy adults) study participants using a convenient sampling technique at Jinella health center, Harar, Eastern Ethiopia, from July 10–August 10, 2022. Five milliliters of venous blood were collected from each study participant, and platelet parameters were analyzed using a Unicel (DxH 800) automated hematologic analyzer. A drop of blood was taken from malaria-suspected patients for blood film preparation. Results between two groups were compared using the Mann–Whitney *U*-test. Spearman's rank correlation coefficient was used to evaluate the relationships between two continuous variables. A *P*-value of < 0.05 was considered statistically significant.

Results: Platelet, plateletcrit, and mean platelet volume of malaria-infected patients were significantly lower as compared with healthy adults (103 x10³cells/μL vs 268 x10³cells/μL, 0.13 fl vs 0.23 fl, and 9.6 fl vs 15.3 fl), respectively). Conversely, platelet distribution width and platelet large cell ratio were higher in malaria-infected patients than healthy adults (19.2% vs 15.3% and 0.35% vs 0.29%), respectively). Parasitemia levels had a moderately inverse correlation with platelet count (*r*= −0.419) and a weakly positive correlation with mean platelet volume (*r*=0.278).

Conclusion: The platelet, plateletcrit, and mean platelet volume of malaria-infected patients were significantly lower as compared with healthy adults. Malaria parasitemia had a moderate inverse correlation with platelet count and a weak positive correlation with mean platelet volume. Thrombocytopenia and alteration of platelet parameters should be considered in malaria patients.

Keywords: adults, malaria, parasitemia level, platelet parameters

Introduction

According to the World Health Organization (WHO), malaria is a potentially fatal condition brought on by parasites that are spread to humans through the bites of infected female *Anopheles* mosquitoes.¹ The two parasite species that represent the greatest threat globally are *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*), which are known to be infectious to humans.² Hematological alterations are among the most frequent malarial consequences, and they have a significant impact on diseases of pathophysiology. Platelets are among the key cell types affected by these frequent

malarial consequences.^{3–5} Furthermore, the pathophysiology of malarial complications can be greatly influenced by the alteration of platelets.^{6,7}

Additionally, the platelet activation changes the shape of platelets, as well as their mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR), and platelet crit (PCT), which are good indicators of their biomass.^{8,9} In 2020, an estimated 241 million malaria cases and 627,000 deaths will have been reported worldwide. Africa bore a disproportionate share of the world's malaria burden.¹ Patients with malaria typically have significantly fewer platelets.¹⁰

The most frequent malaria infection complication is thrombocytopenia, defined as a decreased quantity of platelets,^{4,11,12} and low platelet counts ($<150 \times 10^3$ cells/ μ L) are its defining feature.¹³ People with platelet counts below 150,000 cells/ μ L had a 12–15-fold higher risk of contracting malaria than people with platelet levels above 150,000 cells/ μ L.¹⁴ However, the extent of these changes varies with the degree of malaria endemicity, preexisting hemoglobinopathies, dietary state, demographic variables, and malaria immunity.¹⁰

Peripheral destruction, excessive platelet sequestration in the spleen, and excessive platelet consumption related to the disseminated intravascular coagulation process are the fundamental mechanisms of thrombocytopenia in malaria.¹⁵ Increased secretion of inflammatory cytokines (tumor necrosis factor Alpha (TNF α), Interleukin-1, and Interleukin-10), endothelial cell activation caused by overexpression of cell adhesion molecules like (ICAM-1 and vascular adhesion molecule-1), initiation of the coagulation pathways as a result of platelet consumption, and endothelial damage are all common factors in the entry of the malaria parasite.¹⁶

Additionally, the activation of host immune factors, such as cytokines like TNF α and pro-inflammatory interleukins, oxygen free radicals, and nitric oxide (NO), as well as the production of several toxins, causes damage to the host endothelium and tissues.¹⁷ Previous studies have demonstrated a relationship between parasite density and the seriousness of malarial infection complications.¹⁸ The platelets were also found to be much lower at high parasitemia, and it was reported that increasing *P. falciparum* parasite loads cause a decline in platelet count.¹⁹ Uncertainty exists regarding the degree of platelet parameter alterations that occur during a malarial infection and their underlying biological mechanisms in relation to the level of parasitemia. Little is known about the changes in platelet parameters that occur during malaria infection. Therefore, this study aimed to compare platelet parameters and their correlations with parasitemia among malaria-infected adult patients and apparently healthy adults at Jinella Health Center, Harar, Eastern Ethiopia.

Materials and Methods

Study Design, Area, and Period

A comparative cross-sectional study was conducted in Harar Town, which is one of the regional states of the Federal Democratic Republic of Ethiopia and is located in the eastern part of the country with an elevation of 1885 meters above sea level at latitude 90°12'N and longitude 42°07'E. It is 526 kilometers east of Addis Ababa, Ethiopia's capital city. According to the Harari Regional Health Bureau's 2018 annual report, there were 4 hospitals, 10 health centers, and 20 health posts. Among those ten health centers, the study was conducted at Jinella Health Center from July 10, 2022, to August 10, 2022.

Study Participants

The study population for the malaria-infected group consisted of all adult patients who had been diagnosed with the disease and gave their consent to take part in the study. On the other hand, participants who tested positive for Human Immunodeficiency Viruses (HIV), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus (HCV), pregnant and lactating women, and adults with a history of chronic diseases like hypertension, cardiac disease, and diabetes mellitus were excluded from the malaria-infected group. Additionally, during the study period, adults who were age- and gender-matched and appeared healthy were used as the study population for the comparative group. Healthy adults, including Jinella Health Center employees, volunteers, non-paid blood donors, and patient attendants, were included in the comparison group. Human immunodeficiency viruses, HBsAg and HCV, syphilis, and malaria were screened for, and the health of the comparative group was evaluated using the National Blood Bank Service blood donor questionnaire.

Blood donors with a history of chronic conditions and those who were unwilling to participate in the study were excluded from the comparative group.

Sample Size Determination and Sampling Technique

According to VanVoorhis and Morgan's recommendations, 30 subjects per group are required to find meaningful changes, which might give the study 80% power.²⁰ Then, a total of 186 study participants (93 malaria-infected adults and 93 apparently healthy adults) were recruited using a convenience sampling technique.

Operational Definition

Platelet parameters: platelets and platelet indices (platelet, MPV, PDW, PC, T, and P-LCR).¹⁹

Apparently healthy adults: adult individuals who are in good health and are not suffering from any illness.²¹

Thrombocytopenia: a platelet count of less than 150×10^3 cells / μ L.²²

Levels of malarial parasitemia: high parasitemia (>10 parasites per field), moderate parasitemia (1–10 parasites per field), and low parasitemia (1–100 parasites per 100 fields).²³

Data Collection Procedure and Sampling Processing

Data were collected using a structured questionnaire, which was prepared first in the English language and translated into local languages (Afan Oromo and Amharic) and then back to the English language. Before data collection, data collectors selected patients who qualified for the inclusion criteria and obtained informed consent. Socio-demographics and the history of the patient were collected by administering the questionnaire under the supervision of the principal investigator. Five milliliters of venous blood were collected from both groups by venipuncture according to WHO guidelines²⁴ under aseptic conditions. A drop of blood was taken from the syringe of malaria-susceptible patients for thick and thin blood film (BF) preparation. Then thick and thin BF were prepared according to the WHO guidelines and labeled. The thin films were fixed with absolute methanol, and both thick and thin smears were stained using the 10% Giemsa stain. After the slides were well-dried, malaria infection diagnosis was conducted by detecting and identifying malaria parasites in BF using light microscopy (100x magnification).²⁴

Parasitemia level was determined with thick BF, and species differentiation (confirmation of *Plasmodium* species) was determined with thin BF if doubtful on thick films. The level of asexual parasitemia was calculated using the formula (number of parasites counted/white blood cell counted (WBC)) WBC count/L of blood.²⁵ The smears were reported as negative for malaria only if two consecutive smears were assessed and confirmed negative. The remaining blood sample from malaria-suspected patients and the collected blood from the comparative group were transferred into an ethylene diamine tetraacetic acid (EDTA) tube and gently mixed by inverting the tube 5–6 times to prevent clotting. Then, the samples were analyzed for platelet parameters (platelet count, MPV, PDW, PCT, and P-LCR) using an automated hematology analyzer (UniCel DxH 800).

Statistical Analysis of the Data

After the collection of all the necessary data, it was checked for completeness before entering the Epi-Data software for analysis. Data were entered into Epi-Data version 4.4.2.1, and all data were edited, organized, coded, exported, and analyzed using Statistical Package for Social Sciences (SPSS) version 26 statistical software. The results were presented in graphs and tables. Data for the different continuous platelet parameters were expressed as medians (IQR). The Kolmogorov–Smirnov test was used to check the normal distribution of continuous data. Data between malaria-infected and comparative groups were compared by the Mann–Whitney *U*-test. The Kruskal–Wallis test was applied to compare platelet parameters and levels of parasitemia among malaria-infected adult patients. Spearman's rank correlation coefficient was used to evaluate the relationships between two continuous variables. Categorical variables were compared using the chi-square test. A *p*-value of <0.05 was considered statistically significant.

Data Quality Control

The suitability of the test materials, specimen inspection, and request form review were completed. All laboratory activities were performed with strict adherence to standard operating procedures (SOP). The quality and expiration date

of the methanol used for thin blood fixation and Giemsa stain were checked. Giemsa was used to stain positive malaria slides to ensure the product's quality. The reporting took place following inspections of at least 100 fields following the normal blood film examination technique.

An expert laboratory technologist double-checked any disagreement in blood film results between two laboratory personnel. Strict adherence to the manufacturer's instructions was observed. Quality checks were carried out daily using low, normal, and high control levels to track an instrument's performance over time before beginning sample analysis. The reagent containers were also checked daily for correct connections between the instrument and the reagent containers, sufficient quantity, expiration date, precipitates, turbidity, and particulate matter.

Ethical Consideration

The study was conducted after ethical approval was obtained from the Institutional Health Research Ethics Review Committee (IHRERC), College of Health and Medical Sciences, Haramaya University, with reference number (IHRERC-125-2022). A permission letter was taken from the Jinella Health Center. All study participants gave informed, voluntary, written, and signed consent before data collection. The test results were kept confidential. Additionally, Patients who experienced platelet alterations were also linked to physicians for better management and care. The study was conducted according to the Declaration of Helsinki.

Results

Socio-Demographic Characteristics of Study Participants

A total of 186 study participants (93 malaria-infected adult patients and 93 apparently healthy adults) participated in this study. Among the study participants, the majority of 37 (39.8%) malaria-infected adult patients and 43 (46.2%) apparently healthy adults were aged between 18 and 27 years. In both groups, the fewest number 4 (4.3%) study participants were found between the ages of 58 and 65. In terms of gender, in malaria-infected adult patients and apparently healthy adults, 61 (65.6%) and 59 (63.4%) of the participants were male, respectively. Regarding marital status, most of the study participants—40 (43.0%) malaria-infected adult patients and 53 (57.0%) apparently healthy adults were married. The majority of 53 (57.0%) malaria-infected adult patients were rural residents; however, the majority of 69 (74.2%) apparently healthy individuals were urban residents. Regarding their educational status, 36 (38.7%) of malaria-infected adult patients were able to read and write, and 51 (54.8%) of apparently healthy adult were in college or higher. About 29 (31.2%) of malaria-infected patients were farmers, but 51 (54.8%) of apparently healthy adults were government employees (Table 1).

Distribution of Plasmodium Species and Parasitemia Levels

In the current study, *P. falciparum* 49 (52.7%), *P. vivax* 34 (36.5%), and dual infection 10 (10.8%) were the two plasmodium species identified. In terms of parasitemia levels, 34 (36.6%) had low parasitemia, 30 (32.3%) had moderate parasitemia, and 29 (31.2%) had high parasitemia.

Comparisons of Platelet Parameters Among Study Participants

Platelet parameters of malaria-infected adult patients were compared with those of apparently healthy adults using the Mann–Whitney *U*-test. The median values of platelet count and PCT of malaria-infected patients were significantly lower as compared with apparently healthy adults (103×10^3 cells/ μ L vs 268×10^3 cells/ μ L; $p < 0.001$) and (0.13 fl vs 0.23 fl; $p < 0.001$), respectively. The MPV was also observed to be lower in malaria-infected adult patients (9.6 fl) than in those of apparently healthy adults (15.3 fl), with a statistically significant difference between the two groups ($p < 0.001$). Conversely, the median values of PDW and P-LCR were observed to be higher in malaria-infected adult patients as compared with apparently healthy adults (19.2% vs 15.3%) and (0.35% vs 0.29%), respectively, with a statistically significant difference between the two groups ($p < 0.001$) (Table 2).

Table 1 Socio-Demographic Characteristics of the Study Participants at Jinella Health Center, Harar, Eastern Ethiopia from July-August, 2022(n=93)

Variables	Category	Malaria Patients n (%)	Apparently Healthy n (%)
Age in years	18–27	37 (39.8)	43 (46.2)
	28–37	26 (28.0)	25 (26.9)
	38–47	12 (12.9)	11 (11.8)
	48–57	14 (15.1)	10 (10.8)
	58–65	4 (4.3)	4 (4.3)
Gender	Male	61 (65.6)	59 (63.4)
	Female	32 (34.4)	34 (36.6)
Marital Status	Single	40 (43.0)	36 (38.7)
	Married	40 (43.0)	53 (57.0)
	Divorced	8 (8.6)	3 (3.2)
	Widowed	5 (5.4)	1 (1.1)
Residence	Urban	40 (43.0)	69 (74.2)
	Rural	53 (57.0)	24 (25.8)
Educational Status	Unable to Read and Write	26 (28.0)	7 (7.6)
	Able to Read and Write	36 (38.7)	23 (24.7)
	Preparatory School	16 (17.2)	12 (12.9)
Occupational Status	College and Higher	15 (16.1)	51 (54.8)
	Governmental	12 (12.9)	51 (54.8)
	Merchant	19 (20.4)	11 (11.8)
	Student	16 (17.2)	13 (14.0)
	Farmer	29 (31.2)	9 (9.7)
	Housewife	15 (16.1)	7 (7.5)
	Other	2 (2.2)	2 (2.2)

Table 2 Comparison of Platelet Parameters Among Malaria-Infected Adult Patients and Apparently Healthy Adults at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93) (Mann–Whitney U-Test)

Parameters	Malaria Infected (n) Median (IQR)	Apparently Healthy (n) Median (IQR)	P-value
Platelet ($\times 10^3/\mu\text{L}$)	103(73)	268(98)	< 0.001*
PDW (%)	19.2(3.6)	15.3(2.3)	< 0.001*
MPV (fl)	9.6 (4.3)	15.3(2.2)	< 0.001*
P-LCR	0.35(0.14)	0.29(0.1)	< 0.001*
PCT (fl)	0.13(0.09)	0.23(0.09)	< 0.001*

Notes: *Significant at $p < 0.05$, p-value <0.05 considered statistically significant.

Comparison of Platelet Parameters with Parasitemia Levels

The median platelet count was significantly lower in patients with high parasitemia compared to the low and moderate parasitemia groups ($p < 0.001$). However, the median value of MPV was significantly higher in patients with high parasitemia compared to those with low and moderate parasitemia ($p = 0.001$) (Table 3).

Correlation of Platelet Parameters with Parasitemia Levels

There was a moderate inverse correlation between platelet counts and parasitemia levels ($r = -0.419$, $p < 0.001$) (Figure 1). But there was a weak positive correlation between parasitemia and mean platelet volume ($r = 0.278$, $p = 0.007$). There was a weak inverse correlation between platelet counts and mean platelet volume in malaria-infected adult patients ($r = -0.275$, $p = 0.007$) (Figure 2). However, mean platelet volume had a weakly positive correlation with PDW ($r = 0.275$, $p = 0.008$) and PCT ($r = 0.235$, $p = 0.023$), and a moderately positive

Table 3 Platelet Parameters at Different Parasitemia Levels Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93) (P-value by Kruskal Wallis Test)

Platelet Parameters	Parasitemia Level (Median IQR)			p-value
	Low	Moderate	High	
Platelet($\times 10^3/\pm$)	130.5(147)	112(53)	79(53)	< 0.001*
PDW (%)	20.3(3.8)	18.1(3.2)	18.6(3.7)	0.111
MPV (fl)	9.4(4.1)	8.9(2.7)	12.6(8.9)	0.001*
P-LCR	0.36(0.13)	0.34(0.16)	0.36(0.14)	0.151
PCT (fl)	0.127(0.096)	0.148(0.089)	0.147(0.089)	0.453

Notes: *Significant at $p < 0.05$, p-value < 0.05 considered statistically significant.

correlation with P-LCR among malaria-infected adult patients ($r = 0.532$, $p < 0.001$). In addition to this, PDW had a moderate correlation with PLCR ($r = 0.688$, $p < 0.001$) and a weakly negative correlation with PCT ($r = -0.288$, $p = 0.005$) (Figure 3). A positive correlation was found between platelet count and plateletcrit ($r = 0.4$; p-value < 0.001). Platelet count and platelet distribution width had a negative correlation ($r = -0.5$; $p < 0.001$). No significant correlation was found between platelet count and platelet distribution width.

Prevalence of Thrombocytopenia Among Parasitemia Levels

The overall prevalence of thrombocytopenia in the current study was 77.4 (95% CI: 68.9–85.9). Thrombocytopenia was defined as platelet count $< 150 \times 10^3$ cells/ μ L and further defined as severe if the platelet count was $< 50 \times 10^3$ cells/ μ L, moderate if the platelet count was between $51\text{--}100 \times 10^3$ cells/ μ L, mild if the platelet count was between $101\text{--}150 \times 10^3$ cells/ μ L. Platelet count showed a significant difference among different parasitemia levels ($P = 0.009$) (Table 4).

Discussion

Changes in platelet parameters are considered a hallmark of malaria infection. Most of the time, these changes in malaria infection may be a result of higher levels of parasitemia.²⁶ In this study, the median platelet count was significantly reduced in malaria-infected adult patients as compared to apparently healthy individuals ($P < 0.001$). This result was similar to the finds reported in Thailand,⁸ Colombia,²⁷ Nigeria,²⁸ and Ethiopia.²⁹ The decrease in platelet counts with

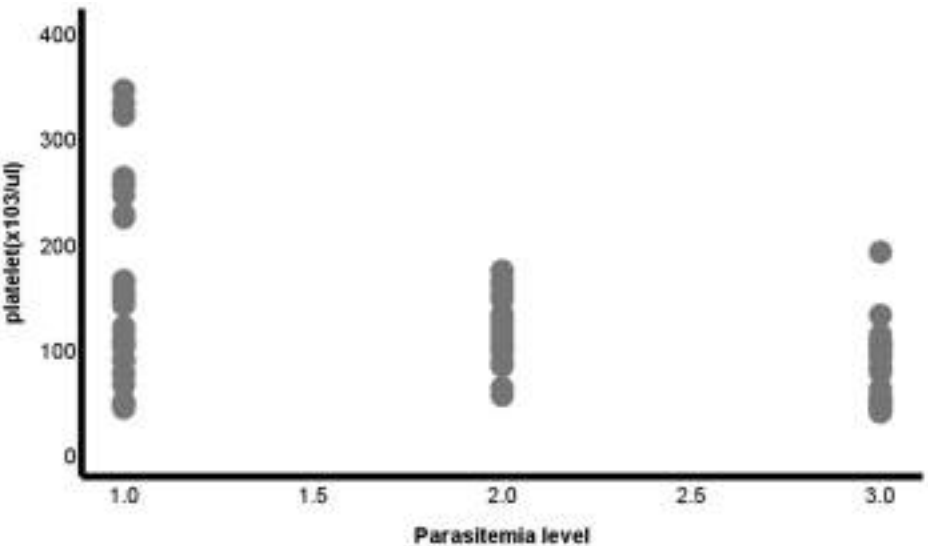


Figure 1 Relationship between platelet count and parasitemia level among malaria-infected adult patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93). **Notes:** NB* 1: Low parasitemia, 2: Moderate parasitemia and 3: Severe parasitemia.

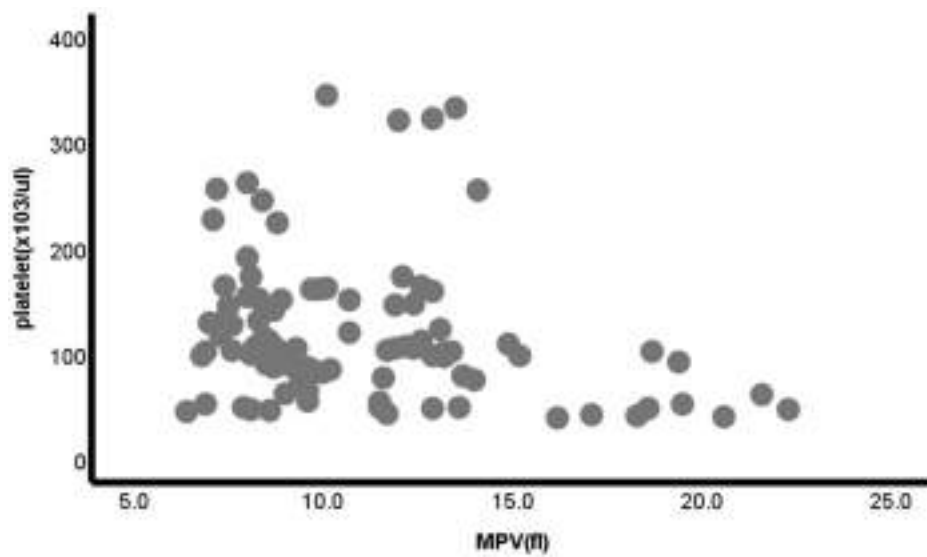


Figure 2 Relationship between platelet count and MPV among malaria-infected adult patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93).

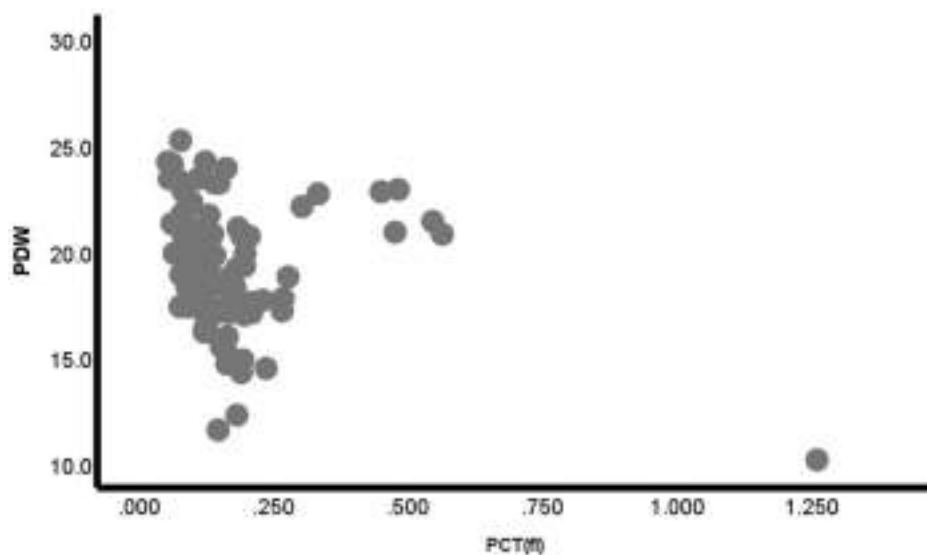


Figure 3 Relationship between PDW and PCT among malaria-infected adult patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93).

malaria might be attributed to platelet degranulation, which ends with the release of platelet factor 4 to kill malaria parasites.^{30,31}

Table 4 Cross Tabulation of Chi-Square Analysis of Platelet Counts Among Parasitemia Levels of Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93)

Parasitemia Level	N	<50×10 ³ /μL	51–100×10 ³ /μL	101–150×10 ³ /μL	151–450 ×10 ³ /μL	χ ² , P-value, and Df
Low	34	5(14.7)	5(14.7)	10(29.4)	14(41.2)	χ ² =17.18 P=0.009 Df=6
Moderate	30	6(20.0)	7(23.3)	11(36.7)	6(20.0)	
High	29	9(31.0)	12(41.4)	7(24.1)	1(3.4)	

The median value of MPV was significantly lower in malaria-infected adult patients than in apparently healthy adults, in agreement with a study conducted in Ghana³² but in contrast with studies conducted in Brazil,¹⁵ Sudan,³³ and Uganda.³⁴ This inconsistency and variation in malaria infection can be explained by the fast splenic and medullary release of huge volumes of platelets in response to the increased demand for these cells.³⁵ The median value of PCT was significantly decreased in malaria-infected adult patients, which was corroborated with previous studies conducted in Thailand,⁸ Ghana,³² and Nigeria.³⁶ Low PCT in malaria may be due to the sensitization of platelets by parasitized red blood cells, resulting in increased platelet sensitivity to adenosine diphosphate (ADP) and better dense-granule secretion. This finding is also explained by concomitant thrombocytopenia.³⁷

The median value of PDW during this study was found to be significantly higher in malaria-infected adult patients, which was in agreement with studies conducted in Colombia,²⁷ Thailand,⁸ and Sudan.^{33,38} The higher PDW values in malaria can be explained by the bone marrow formation of megakaryocytes to make amends for the low absolute platelet count during acute malaria infection.³⁹ However, this finding was in contrast with a study conducted in Ghana.³² This discordant result was attributed to several factors that will result in changes in PDW, like the recruitment of multiple ploidy classes of megakaryocytes.⁴⁰

Regarding P-LCR, the median significantly increased in malaria-infected adult patients, which was compatible with the study conducted in Nigeria³⁶ and Sudan.³⁸ This could be because larger platelets are typically younger and contain more intracellular granules, resulting in a higher thrombogenic potential.⁴¹ In contrast to this study, a study conducted in Ghana³² showed that the P-LCR was statistically lower than that of a comparative group. Previously, variations in the P-LCR value could be attributed to thrombopoietin suppression by TNF- α , which can inhibit thrombopoiesis and, in fact, reduce the number of juvenile thrombocytes. Thus, elevation of TNF- α in malaria infections is thought to down-regulate Interleukin-10, which inhibits erythropoietin and possibly thrombopoietin biosynthesis and its activity.⁴²

There were significant differences in platelet count and MPV between malaria parasitemia levels (P value <0.001 and 0.007, respectively). The platelet count was notably reduced in patients with high parasitemia compared to those in the low and moderate parasitemia groups. Malaria parasitemia also had a moderately negative correlation with platelet count ($r = -0.419$, $p < 0.001$) (Table 5). Many previous studies reported similar correlational statistics between platelet count and malaria parasitemia,^{29,43,44} but this finding was in contrast to the study conducted in Brazil.²⁶ The decrement of platelets may be due to the role of von Willebrand factor (VWF) multimers in promoting platelet-mediated IE (infected erythrocyte) cytoadherence, and it was proposed that this mechanism may be particularly important during the first stages of malaria infection.⁴⁵ Additionally, platelets in patients with malaria infection express Toll-like receptors (TLRs), which release prepackaged inflammatory mediators like NO, a key mediator of platelet homeostasis. Then, a decreased bioavailability of NO in patients with severe malaria may contribute to increased platelet activation and consumption as parasitemia increases.¹⁹

Table 5 Correlation of Platelet Parameters Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia, 2022 (n=93) (Spearman's Rank-Order Correlation)

Correlation Between Parameters		Correlation Coefficient	
		Rh0	p
Platelet count	Parasitemia level	-0.419	<0.001
	MPV	-0.275	0.007
MPV	Parasitemia level	0.278	0.007
	PDW	0.275	0.008
	PCT	0.235	0.023
	PLCR	0.532	0.001
PDW	PCT	-0.288	0.005
	PLCR	0.688	<0.001

The MPV value was found to be higher in patients with high parasitemia compared to those in the low and moderate parasitemia groups. There was also a weak direct correlation between parasitemia levels ($r = 0.278$, $p = 0.007$) in contrast with a study conducted in Sudan.⁴⁴ This variability could be due to the platelet size being larger than average as parasitemia increases, which is typically an indication that the patient's body is producing too many immature platelets.⁴⁶

In the present study, there was a moderately weak inverse correlation between platelet count and MPV ($r = -0.257$; $p = 0.007$) which was in accordance with other studies.^{43,47,48} When platelets decrease in number, an early release of platelets from the bone marrow occurs. These early released platelets are larger in size, and the stimulation of megakaryocytes within the marrow by thrombopoietin in response to low platelets produces larger platelets as their nucleus becomes hyper-lobulated with the next polymer.²⁷ The MPV had a weak positive correlation with PDW ($r = 0.275$; $p = 0.008$) and PCT ($r = 0.235$; $p = 0.023$) which was in accordance with the study conducted in Mangalore.⁴³ Mean platelet volume reflects platelet size, whereas PDW reflects variability in platelet size and is taken into account as a marker of platelet function and activation. So, a high PDW means platelet size varies greatly, which is a clue that there's platelet activation and has been related to vascular diseases.⁴⁹ This convergence of MPV and PDW changes may be useful for detecting malaria infection during the first stage of gametocyte sequestration in bone marrow.⁵⁰

In addition to this, there was a moderate correlation between MPV and P-LCR ($r = 0.532$; $p = 0.001$) which was agreed with in a study conducted in Nigeria.³⁶ Since P-LCR is an indicator of circulating larger platelets (> 12 fL), as MPV becomes higher, there's a tendency for P-LCR to be increased.⁵¹ Platelet distribution width had a weak negative correlation with PCT ($r = -0.288$; $p = 0.005$), which is the product of MPV and platelet count and represents the percentage of space platelets occupy within the blood.⁵² On the other hand, PDW had a moderately positive correlation with P-LCR ($r = 0.688$; $P < 0.001$). A high P-LCR or PDW may indicate peripheral immune destruction of platelets.⁵³

In the current study, the prevalence of thrombocytopenia was 77.4 (95% CI: 68.9–85.9). Comparable results were found in studies conducted in India (71%),⁵⁴ Pakistan (70%),⁵⁵ and Gujarat, India (82%).⁵⁶ But the prevalence of thrombocytopenia was found to be higher than in similar studies conducted in Saudi Arabia (67.5%),⁵⁷ Indonesia (33%),⁵⁸ Aden (42.9%),⁵ and Turkey (47%),⁵⁹ and Ghana (47%).⁶⁰

On the contrary, the prevalence of thrombocytopenia in this study was lower than in the studies conducted in Karnataka, India (89.0%),⁴⁸ and Karamsad, India (90%).⁶¹ This discrepancy might be due to the study designs used, sample size variation between different studies, malaria distribution across different countries, or the type of study participants. Another possible cause for this discrepancy might be due to splenic pooling of platelets and antibody-mediated platelet destruction.^{33,36,37}

Conclusion and Recommendation

It is evident from the results of our current study that the median values of platelet count, MPV, and PCT of adult malaria-infected patients were significantly lower as compared with apparently healthy adults. Besides, malaria parasitemia level had a moderate inverse correlation with platelet count and a weak positive correlation with MPV. In addition, the documented low levels of platelet count and variation in platelet parameters in malaria-positive cases have significant value in supporting the clinical diagnosis of malaria. The causes of thrombocytopenia and alteration of platelet indices should be taken into consideration when comparing findings in malaria patients. Whether or not our findings may be utilized in monitoring the effect of malaria treatment, more work is required to relate these findings to the presence of any parasite component that may be useful to realize the mechanism of platelet parameter alterations.

Abbreviations

BF, Blood Film; EDTA, Ethylene Diamine Tetra Acetic Acid; **Hgb, Hemoglobin;** ICAM-1, Intravascular cell adhesion molecule-1; MPV, Mean Platelet Volume; NO, Nitric Oxide; PCT, Plateletcrit; PDW, Platelet Distribution Width; P-LCR, Platelet Large Cell Ratio; *PF, Plasmodium Falciparum*; *PV, Plasmodium Vivax*; TNF α , Tumor Necrosis Factor-alpha; VWF, Von Willebrand factor; WHO, World Health Organization.

Acknowledgments

We would like to express our heartfelt gratitude to the laboratory technicians and all staff members of Jinella Health Center for their unreserved support in blood sample collection, processing, and use of their laboratory facilities. In addition, we would also like to acknowledge the study participants and data collectors.

Author Contributions

All authors made a significant contribution to the work reported in the conception, study design, execution, acquisition, analysis, and interpretation of data; and took part in drafting, revising, and critically reviewing the manuscript. All authors have agreed on the approval of the final manuscript to be published in the current journal and to be accountable for all aspects of the work.

Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. World Health Organization. *World Malaria Report 2021*. Geneva, Switzerland: World Health Organization; 2021.
2. World Health Organization. *Eliminating Malaria*. World Health Organization; 2016.
3. van Wolfswinkel M, Vliegthart-Jongbloed K, de Mendonça Melo M, et al. Predictive value of lymphocytopenia and the neutrophil-lymphocyte count ratio for severe imported malaria. *Malar J*. 2013;12(1):1–8. doi:10.1186/1475-2875-12-101
4. Khan S, Abbass Y, Marwat M. Thrombocytopenia as an indicator of malaria in adult population. *Malar Res Treat*. 2012;2012:1–4. doi:10.1155/2012/405981
5. Bakhubaira S. Hematological parameters in severe complicated Plasmodium falciparum malaria among adults in Aden. *Turkish J Hematol*. 2013;30(4):394. doi:10.4274/Tjh.2012.0086
6. Mohandas N, An X. Malaria and human red blood cells. *Med Microbiol Immunol*. 2012;201(4):593–598. doi:10.1007/s00430-012-0272-z
7. Cox D, McConkey S. The role of platelets in the pathogenesis of cerebral malaria. *Cell Mol Life Sci*. 2010;67(4):557–568. doi:10.1007/s00018-009-0211-3
8. Tangvarasittichai O, Srikong M, Tangvarasittichai S. Platelet count and platelet indices used as potential markers for first malaria infection diagnosis. *Int J*. 2016;8(10):1454–1458.
9. Akpinar I, Sayin M, Gursay Y, et al. Plateletcrit. *Herz*. 2014;39(1):142–148. doi:10.1007/s00059-013-3798-y
10. Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. *Malar J*. 2014;13(1):1–7. doi:10.1186/1475-2875-13-218
11. Moulin F, Lesage F, Legros A, et al. Thrombocytopenia and Plasmodium falciparum malaria in children with different exposures. *Arch Dis Child*. 2003;88(6):540–541. doi:10.1136/adc.88.6.540
12. Abid M, Muhammad Y. Thrombocytopenia: a predictor of malaria among febrile patients in Liberia. 2005.
13. Bayleyegn B, Asrie F, Yalew A, Woldu B. Role of platelet indices as a potential marker for malaria severity. *J Parasitol Res*. 2021;2021. doi:10.1155/2021/5531091
14. Erhart L, Yingyuen K, Chuanak N, et al. Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. *Am J Trop Med Hyg*. 2004;70(1):8–14. doi:10.4269/ajtmh.2004.70.8
15. Leal-Santos F, Silva S, Crepaldi N, et al. Altered platelet indices as potential markers of severe and complicated malaria caused by Plasmodium vivax: a cross-sectional descriptive study. *Malar J*. 2013;12(1):1–6. doi:10.1186/1475-2875-12-462
16. D'souza J, Jayaprakash C, D'souza P, Abraham S, Suresh S, Shrinath M. Comparative hematological changes in malarial infection by P. vivax and P. falciparum: observations from the endemic region of Mangalore, India. *Int J Appl Res*. 2017;3(6):179–183.
17. Luzzatto L. Genetics of red cells and susceptibility to malaria; 1979.
18. Tangpukdee N, Krudsood S, Kano S, Wilairatana P. Falciparum malaria parasitemia index for predicting severe malaria. *Int J Lab Hematol*. 2012;34(3):320–327. doi:10.1111/j.1751-553X.2011.01398.x
19. Kotepui M, Piwklam D, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effects of malaria parasite density on blood cell parameters. *PLoS One*. 2015;10(3):e0121057. doi:10.1371/journal.pone.0121057
20. VanVoorhis CW, Morgan BL. Understanding power and rules of thumb for determining sample sizes. *Tutor Quant Methods Psychol*. 2007;3(2):43–50. doi:10.20982/tqmp.03.2.p043
21. Awulachew E, Diriba K, Anberbir S. Hematological and Immunological parameters in apparently healthy people in Ethiopia: systematic review and meta-analysis; 2020.
22. Roberts I, Stanworth S, Murray N. Thrombocytopenia in the neonate. *Blood Rev*. 2008;22(4):173–186. doi:10.1016/j.blre.2008.03.004
23. Al-Salahy M, Shnawa B, Abed G, Mandour A, Al-Ezzi A. Parasitaemia and its relation to hematological parameters and liver function among patients malaria in Abs, Hajjah, Northwest Yemen. *Interdiscip Perspect Infect Dis*. 2016;2016:1–5. doi:10.1155/2016/5954394
24. World Health Organization. *Guidelines on Drawing Blood: Best Practices in Phlebotomy*. World Health Organization; 2010.
25. Cserti M, Dzik H. The ABO blood group system and Plasmodium falciparum malaria. *Blood*. 2007;110(7):2250–2258.
26. Dos-Santos JCK, Silva-Filho JL, Judice CC, et al. Platelet disturbances correlate with endothelial cell activation in uncomplicated Plasmodium vivax malaria. *PLoS Negl Trop Dis*. 2020;14(7):e0007656. doi:10.1371/journal.pntd.0007656
27. Martínez-Salazar EL, Tobón-Castaño A. Platelet profile is associated with clinical complications in patients with vivax and falciparum malaria in Colombia. *Rev Soc Bras Med Trop*. 2014;47:341–349. doi:10.1590/0037-8682-0078-2014

28. Adias TC, Bariweni M, Dick PD, et al. Effects of plasmodium falciparum on haematological parameters on patients treated with artesunate in the Niger delta of Nigeria. *Int J Med Sci Public Health*. 2013;1(12):12.
29. Awoke N, Arota A. Profiles of hematological parameters in Plasmodium falciparum and Plasmodium vivax malaria patients attending Tercha General Hospital, Dawuro Zone, South Ethiopia. *Infect Drug Resist*. 2019;12:521. doi:10.2147/IDR.S184489
30. Kho S, Barber BE, Johar E, et al. Platelets kill circulating parasites of all major Plasmodium species in human malaria. *Blood*. 2018;132(12):1332–1344.
31. Peprah S, Ogwang MD, Kerchan P, et al. Mean platelet counts are relatively decreased with malaria but relatively increased with endemic Burkitt Lymphoma in Uganda, Tanzania, and Kenya. *Br J Haematol*. 2020;190(5):772–782. doi:10.1111/bjh.16700
32. Aninagyei E, Adu P, Rufai T, et al. Effect of asymptomatic Plasmodium falciparum parasitaemia on platelets thrombogenicity in blood donors. *Indian J Hematol Blood Transfus*. 2021;37(4):632–639. doi:10.1007/s12288-020-01390-w
33. Ali EA, Abdalla TM, Adam I. Platelet distribution width, mean platelet volume and haematological parameters in patients with uncomplicated Plasmodium falciparum and P. vivax malaria. *F1000Research*. 2017;6:865.
34. Muwonge H, Kikomeko S, Sembajje LF, Seguya A, Namugwanya C. How reliable are hematological parameters in predicting uncomplicated Plasmodium falciparum malaria in an endemic region? *Int Sch Res Notices*. 2013;2013:9.
35. Becchi C, Al Malyan M, Fabbri L, Marsili M, Boddi V, Boncinelli S. Mean platelet volume trend in sepsis: is it a useful parameter? *Minerva Anesthesiol*. 2006;72(9):749–756.
36. Yusuf AA, Abdullahi SA, Idris IM, Jobbi YDA. Platelet count and indices in acute uncomplicated Malaria in Kano, Nigeria. *Niger J Clin Sci*. 2019;16(1):46. doi:10.4103/njbs.njbs_21_18
37. Prasad R, Das B, Pengoria R, Mishra OP, Shukla J, Singh TB. Coagulation status and platelet functions in children with severe falciparum malaria and their correlation of outcome. *J Trop Pediatr*. 2009;55(6):374–378. doi:10.1093/tropej/fmp028
38. Elsayid M, Elbasheer M, Othman AA, Belal RH, Elfaki T. Estimation of complete blood count and platelets indices in Sudanese patients with malaria. *Am J Res Commun*. 2017;5(1):29–38.
39. Chandra S, Chandra H. Role of haematological parameters as an indicator of acute malarial infection in Uttarakhand state of India. *Mediterr J Hematol Infect Dis*. 2013;5(1):e2013009. doi:10.4084/mjhid.2013.009
40. Hamid T, Eldin FA, Elnasri HA. Effect of Malaria Parasite Infection on Platelet Parameters (Platelets count, mean platelets volume and platelets distribution width) among Malaria Patients in Khartoum State. *Ann Biol Res*. 2016;7(5):18–23.
41. Desai K, Patel K, Shah M, et al. A study of platelet volume indices (PVI) in patients of coronary artery disease and acute myocardial infarction in tertiary care hospital. *Int J Adv Res*. 2013;1:185–191.
42. Othoro C, Lal AA, Nahlen B, Koech D, Orago AS, Udhayakumar V. A low interleukin-10 tumor necrosis factor- α ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. *J Infect Dis*. 1999;179(1):279–282. doi:10.1086/314548
43. Kumar N. Correlation of type of species and parasite density in malaria with platelet count, mean platelet volume and platelet distribution width. *J Evol Med Dent Sci*. 2016;5(89):6622–6625. doi:10.14260/Jemds/2016/1498
44. Mohamedahmed KA, Ahmed ZA, Nour BYM, Abakar AD, Babker AM. Impact of sever Plasmodium falciparum infection on platelets parameters among Sudanese children living in Al-Jazira state. *Int J Clin Biomed Res*. 2020;5–9. doi:10.31878/ijcbr.2020.62.02
45. Pain A, Ferguson DJ, Kai O, et al. Platelet-mediated clumping of Plasmodium falciparum-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc Natl Acad Sci USA*. 2001;98(4):1805–1810. doi:10.1073/pnas.98.4.1805
46. Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemonia H, Dymicka-Piekarska V. Mean Platelet Volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediators Inflamm*. 2019;2019:9213074. doi:10.1155/2019/9213074
47. Coelho HCC, Lopes SC, Pimentel JPD, et al. Thrombocytopenia in Plasmodium vivax malaria is related to platelets phagocytosis. *PLoS One*. 2013;8(5):e63410. doi:10.1371/journal.pone.0063410
48. Gupta P, Guddattu V, Saravu K. Characterization of platelet count and platelet indices and their potential role to predict severity in malaria. *Pathog Glob Health*. 2019;113(2):86–93. doi:10.1080/20477724.2019.1600855
49. Vagdatli E, Gounari E, Lazaridou E, Katsibourlia E, Tsikopoulou F, Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia*. 2010;14(1):28.
50. Helvacioğlu C, Baghaki S, Bibata BB, Yıldırım Karaca S, Doğan K. Can platelet indices be of value in pregnant women with malaria? *J Obstet Gynaecol*. 2022;42:1–5.
51. Budak YU, Polat M, Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. *Biochemia medica*. 2016;26(2):178–193. doi:10.11613/BM.2016.020
52. Chandrashekar V. Plateletcrit as a screening tool for detection of platelet quantitative disorders. *J Hematol*. 2013;2(1):22–26.
53. Bain BJ, Bates I, Laffan MA, Lewis SM. *Dacie and Lewis Practical Haematology: Expert Consult: Online and Print*. Elsevier Health Sciences; 2016.
54. Surve KM, Kulkarni AS, Rathod SG, Bindu RS. Study of haematological parameters in malaria. *Int J Res Med Sci*. 2017;5(6):2552–2557. doi:10.18203/2320-6012.ijrms20172446
55. Memon AR, Afsar S. Thrombocytopenia in hospitalized malaria patients. *Pak J Med Sci Q*. 2006;22(2):141.
56. Patel A, Jain S, Patel B, Modi B. Hematological changes in P. falciparum & P. vivax malaria. *NJMR*. 2013;3(02):130–133.
57. Bashawri LA, Mandil AA, Bahnassy AA, Ahmed MA. Malaria: hematological aspects. *Ann Saudi Med*. 2002;22(5–6):372–376. doi:10.5144/0256-4947.2002.372
58. Aundhakar S, Prajapati P, Prajapati S, et al. Study of clinical and hematological profile of Plasmodium vivax malaria in a tertiary care hospital in Western Maharashtra. *Int J Sci Study*. 2017;5(3):257–260.
59. Mert A, Ozaras R, Tabak F, Bilir M, Ozturk R, Aktuglu Y. Malaria in Turkey: a review of 33 cases. *Eur J Epidemiol*. 2003;18(6):579–582. doi:10.1023/A:1024648902848
60. Sakzabre D, Asiamah EA, Akorsu EE, et al. Haematological profile of adults with malaria parasitaemia visiting the Volta Regional Hospital, Ghana. *Adv Hematol*. 2020;2020:1–6. doi:10.1155/2020/9369758
61. Rajani D, Khatri D, Vaishnav D. Malaria: recent trends manifested as thrombocytopenia. *Int J Med Sci Educ*. 2016;3(1):7–10.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Outcomes of Hydroxyurea Accessed via Various Means and Barriers Affecting Its Usage Among Children with Sickle Cell Anaemia in North-Western Tanzania

Emmanuela E Ambrose, Benson R Kidenya, Mwesige Charles, Joyce Ndunguru, Agnes Jonathan, Julie Makani, Irene K Minja, Paschal Ruggajo & Emmanuel Balandya

To cite this article: Emmanuela E Ambrose, Benson R Kidenya, Mwesige Charles, Joyce Ndunguru, Agnes Jonathan, Julie Makani, Irene K Minja, Paschal Ruggajo & Emmanuel Balandya (2023) Outcomes of Hydroxyurea Accessed via Various Means and Barriers Affecting Its Usage Among Children with Sickle Cell Anaemia in North-Western Tanzania, Journal of Blood Medicine, , 37-47, DOI: [10.2147/JBM.S380901](https://doi.org/10.2147/JBM.S380901)

To link to this article: <https://doi.org/10.2147/JBM.S380901>



© 2023 Ambrose et al.



Published online: 21 Jan 2023.



Submit your article to this journal



Article views: 518



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Outcomes of Hydroxyurea Accessed via Various Means and Barriers Affecting Its Usage Among Children with Sickle Cell Anaemia in North-Western Tanzania

Emmanuela E Ambrose¹, Benson R Kidenya², Mwesige Charles³, Joyce Ndunguru⁴, Agnes Jonathan⁴, Julie Makani⁴, Irene K Minja^{4,5}, Paschal Ruggajo^{4,6}, Emmanuel Balandya^{4,7}

¹Department of Paediatrics and Child Health, Catholic University of Health and Allied Sciences, Mwanza, Tanzania; ²Department of Biochemistry and Molecular Biology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania; ³Department of Laboratory Services, Bugando Medical Centre, Mwanza, Tanzania; ⁴Department of Haematology and Blood Transfusion, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; ⁵Department of Restorative Dentistry, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; ⁶Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; ⁷Department of Physiology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

Correspondence: Emmanuela E Ambrose, Department of Paediatrics and Child Health, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania, Tel +255789733833, Fax +255282500799, Email emmanuelaambrose@gmail.com

Purpose: To assess clinical and haematological outcomes of Hydroxyurea accessed via various access means and uncover the barriers to its utilization in children with Sickle cell anaemia (SCA), North-western Tanzania.

Patients and Methods: A retrospective study was conducted between October 2020 and April 2021 at Bugando Medical Centre (BMC) through review of medical files to compare the clinical and haematological outcomes among children with SCA at baseline and followed up retrospectively for at least one year of hydroxyurea utilization, accessed via cash, insurance and projects. Subsequently, a cross-sectional survey was conducted among parents and caregivers to ascertain the barriers to access of hydroxyurea via the various means. The p-values <0.05 were considered statistically significant.

Results: We identified 87 children with SCA who were on hydroxyurea for at least one year. The median age at baseline (before hydroxyurea) was 99 [78–151] months, and 52/87 (59.8%) were male. Compared to baseline, there was a significant reduction in proportion of patients reporting vaso-occlusive crisis, admissions and blood transfusions, a significant increase in Haemoglobin and mean corpuscular volume, conversely a significant reduction in absolute neutrophil and reticulocytes to both insurance and project participants. There was no significant change in most of these parameters among patients who accessed hydroxyurea via cash. Further, a total of 24/87 (27.6%) participants reported different barriers to access of hydroxyurea, where 10/24 (41.7%) reported hydroxyurea to be very expensive, 10/24 (41.7%) reported insurance challenges, and 4/21 (16.6%) reported unavailability of the drug.

Conclusion: The paediatric patients utilizing hydroxyurea accessed via insurance and projects, but not cash, experienced significant improvement in the clinical and haematological outcomes. Several barriers for access to hydroxyurea were observed which appeared to impact these outcomes. These findings call for concerted efforts to improve the sustainable access to hydroxyurea among all patients with SCA.

Keywords: sickle cell anaemia, hydroxyurea, access, outcomes, North-western Tanzania

Introduction

Sickle cell anaemia (SCA) is the most common genetic disorder of the red blood cells (RBCs) characterized by a single base mutation on the 6th codon of the β globin gene, resulting in substitution of amino acid valine for glutamic acid.¹ This change causes the RBCs to change to a sickle shape under hypoxic conditions due to polymerization of the haemoglobin molecules,^{1,2} which is the cause for the multitude of complications in SCA such as cerebro-vascular accidents, vaso-occlusive crisis (VOC), acute chest syndrome, spleen sequestration and end organ damage.²

Worldwide, Tanzania ranks fourth among countries with the highest annual births of individuals with SCA.³ The high disease burden is mostly seen in regions along the Lake Victoria zone of the country.^{3–6} Subsequently, an overwhelming number of patients with a myriad of SCA-related complications attend at the Bugando Medical Centre (BMC), the zonal tertiary referral hospital.⁷

Hydroxyurea is the first disease modifying agent to be approved for use in SCA.⁸ It does so by inducing foetal haemoglobin (HbF) production, decreasing intracellular sickle haemoglobin (HbS) polymerization within RBCs, decreasing RBCs adhesion to endothelium,⁹ and increasing release of the potent vasodilator nitric oxide.⁹ The use of hydroxyurea has been shown to reduce the frequency of pain crisis, acute chest syndrome and blood transfusions in patients with SCA.⁹ The overall safety and benefits of hydroxyurea have been demonstrated in diverse SCA populations, including children with SCA in sub-Saharan Africa (SSA).^{10–12} These benefits however depend of sustained exposure to the medication.¹³ The use of hydroxyurea in SCA is recommended from as early as 9 months of age and should generally be continued throughout life.¹⁴

While the use of hydroxyurea is common in the developed countries, most patients with SCA in SSA are not able to access the medication. In this setting, several challenges have been reported spanning from the introduction of hydroxyurea to utilisation by the patients. These challenges include bottlenecks in registration of hydroxyurea as a drug for patients with SCA, shortage of healthcare workers, high costs of the medication and misconceptions regarding use of hydroxyurea for SCA among patients, caregivers and prescribers.^{15–17} In order to improve the utilisation of hydroxyurea among patients with SCA in SSA, it is imperative to ensure that the drug is available and affordable to all in need and that healthcare workers caring for patients with SCA have proper training on prescription of hydroxyurea.^{15,16}

Along the shores of Lake Victoria, hydroxyurea is obtained either through self-purchase out-of-pocket, health insurance services, different donations by private/non-governmental organizations or through research projects. This applies to most other parts of the country as well since there is currently no universal health insurance policy. All these means of access pose different challenges with regard to eligibility and therefore number of patients that can be accommodated, age at initiation of access to hydroxyurea, and continuity of access to the drug. Since the goal is to ensure as many patients as possible are initiated on hydroxyurea early on and continue to take the medication for life with minimal interruptions, it is imperative to evaluate the clinical effects and challenges associated with the use of hydroxyurea accessed via the different means.

The present study therefore evaluated the clinical and haematological outcomes and barriers for access to hydroxyurea via cash, insurance and projects among paediatric SCA patients attended at the BMC in Mwanza, Tanzania. Insights from this study will inform policy on the best way or combination of ways towards achieving universal and optimal access to hydroxyurea among patients with SCA as the standard of care in Tanzania and other countries in the developing world.

Materials and Methods

Study Area

This study was conducted at the department of paediatrics and child health of the BMC in Tanzania. BMC is a 900-bed consultant and teaching hospital located along the shores of Lake Victoria in Mwanza region, and serves as the tertiary referral hospital for the Lake Victoria and Western zones of the United Republic of Tanzania. It has a catchment area of eight regions including Mwanza, Geita, Kigoma, Kagera, Mara, Shinyanga, Simiyu and Tabora, serving an estimated population of 16 million people. The department of paediatrics and child health has a capacity of 128 beds. The department is subdivided into inpatient and outpatient sections. From January 2020 to June 2020, a total of 300 children were admitted with SCA-related complications. Paediatric SCA clinic at BMC serves 80 patients per week aged 6 months to 12 years. Majority of children with SCA seen at the clinic are diagnosed with sickling test or sickle SCAN point-of-care test (Biomedomics Inc., USA) and confirmed by haemoglobin electrophoresis (HbE), Isoelectric focusing (IEF) or high performance liquid chromatography (HPLC). Routine care for patients with SCA include folic acid and Pen V (available to all patients) and also hydroxyurea if available through projects or if the parents can afford to subscribe to health insurance or purchase the drug out-of-pocket. During the study period, approximately 80% of all patients with SCA seen at BMC were children, and only about 26% of them were on hydroxyurea.

Study Design

The study was conducted between October 2020 and April 2021. The first part of the study involved retrospective evaluation of the medical files of paediatric patients with SCA who attended at the hospital between June 2019 and October 2020 to ascertain who were on hydroxyurea, duration on the drug, and outcomes/parameters extracted from the patients' medical files prior initiation of hydroxyurea. After written informed consent was obtained from the parents or guardians, and assent from children where appropriate, patients who were on hydroxyurea for a least one year were enrolled into a cross-sectional survey to ascertain the clinical outcomes after hydroxyurea initiation, means of access (16 patients-cash, 43 patients-insurance or 28 patients- projects) and barriers to access of hydroxyurea. Demographic and clinical information as well as information on hydroxyurea access was collected using a standardized questionnaire. A venous blood sample was then collected for assessment of the complete blood count (CBC), reticulocyte count and HbF at the time of enrolment.

Ethical Issues

Ethical clearance for the research proposal was sought from the joint Bugando Medical Centre and Catholic University of Health and Allied Sciences (BMC/CUHAS) ethics and review committee with a certificate number CREC/437/2020. Furthermore, written informed consent was obtained from the parents of children with SCA who participated in the study after they clearly understood the objectives of the study which was presented using the information sheet and consent form. Assent was sought for children above the age of 10 years. The informed consent forms were then stored safely together with the data collection forms in a safe location. All activities performed in this study comply with the Declaration of Helsinki.

Laboratory Methods

Two millilitres (mls) of peripheral venous blood were collected in ethylene-diamine-tetra-acetic acid (EDTA) tubes from all children on hydroxyurea for at least one year at the time of enrolment into the cross-sectional survey. A complete blood count with reticulocyte count was performed using the Cell-Dyn 3700 Haematology Analyzer (Abbott Diagnostics, Lake Bluff, IL, USA). The identification of HbA, HbS, HbF and other haemoglobin variants was done using HbE (Biotec Fisher Filipo) or IEF (GE Healthcare Bio Sciences AB, Bjorktan 30,751 84 Uppsala Sweden). The identification and quantification of HbA, HbS, HbF and other variants was done using high-performance liquid chromatography (HPLC) using the Variant I Haemoglobin Testing System (Bio-Rad, Laboratories, Inc., Hercules, CA, USA). Patients with HbS and no detectable HbA were considered to have SCA. Patients with HbS and low levels of detectable HbA on HPLC or light HbA band on HbE/IEF were considered to have sickle beta plus thalassemia (HbS β + thal) and were excluded from the study. All tests were performed under the experimental conditions specified by the manufacturers.

Data Analysis

Data was entered into Microsoft excel database and analyzed by using STATA version 16 (Stata Corp, College Station, Texas, USA). Descriptive statistics were summarized in frequencies and proportions (percent) (for categorical variables), means with standard deviations or medians and interquartile ranges (for continuous variables, depending on distribution), and were presented in tables and figures. The prevalence of clinical outcomes (VOC, admissions, blood transfusion) were compared before and after hydroxyurea initiation using two-sample test of proportions. Haematological outcomes (Hb, MCV and ANC) before and after hydroxyurea initiation were compared using Wilcoxon matched-pairs signed-ranks test for continuous variables. P-values of <0.05 were considered statistically significant.

Results

Enrolment

From October 2020 to April 2021, a total of 132 children with SCA were screened for eligibility. Of these, 87 children were on hydroxyurea for more than one year. Past medical history and medical files were reviewed. The median age of patients prior to hydroxyurea initiation was 99 [78–151] months and the median duration on hydroxyurea was 22.6 [16.6–37.2] months at study enrolment. From the 87 participants, 16/87 (18.4%) were accessing hydroxyurea through cash, 43/87 (49.4%) through health insurance coverage, and 28/87 (32.2%) through projects (Figure 1).

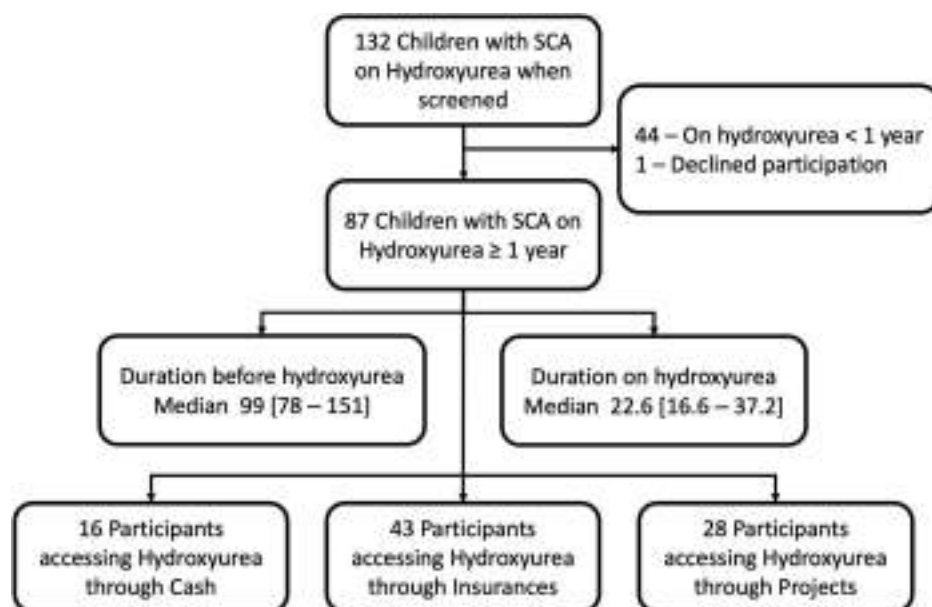


Figure 1 Enrolment flowchart of sickle cell anaemia participant on Hydroxyurea from October 2020 to April 2021.

Baseline History and Clinical Characteristics of 87 Children with SCA on Hydroxyurea for at Least One Year

The median age at enrolment in the cross-sectional survey for the 87 children with SCA patients prior to hydroxyurea initiation was 99 [78–151] months and the median duration on hydroxyurea was 22.6 [16.6–37.2] months at study enrolment, 52/87 (59.8%) were male. Majority of the children were from Mwanza region 66/87 (75.6%). Almost all children reported different complications encountered before initiation on hydroxyurea. Majority of participants had reported to have had malaria 76/87 (87.4%) and 55/87 (63.2%) had acute severe pulmonary event (acute chest syndrome or pneumonia). Stroke was reported by 22/87 (25.3%) participants (Table 1).

Table 1 Baseline Characteristics of Sickle Cell Anaemia (SCA) Participants

Variable		n (Median/%)
A: Personal characteristics		
Age in months (median)		99 [78–151]
Sex	Female	35 (40.2)
School grade	Secondary	12 (13.8)
	Primary	55 (63.2)
	Not studying currently	20 (23.0)
Tribe	Sukuma	21 (24.1)
	Kurya	18 (20.7)
	Others	48 (55.2)
Region	Mwanza	66 (75.8)
	Mara	10 (11.5)
	Other	12 (12.7)

(Continued)

Table I (Continued).

Variable		n (Median/%)
Malaria history	Yes	76 (87.4)
Dactylitis	Yes	60 (69.0)
Splenic sequestration	Yes	43 (49.4)
Acute Chest Syndrome	Yes	55 (63.2)
Vaso Occlusive Crisis	Yes	86 (98.9)
Sepsis	Yes	78 (89.6)
Stroke	Yes	22 (25.3)
Hospitalization	≤ 5	29 (23.3)
	6–10	10 (11.5)
	>10	43 (49.4)
Lifetime Blood Transfusion	≤ 5	45 (51.7)
	6–10	12 (13.8)
	>10	20 (22.9)
How Hydroxyurea is obtained	Cash	16 (18.4)
	Insurance	43 (49.4)
	Ongoing Projects	28 (32.2)
Pallor	Present	44 (50.6)
Icterus	Present	38 (43.7)
Nails	Abnormal	4 (4.6)
Spleen	Palpated	11 (12.6)
	Splenectomy	3 (3.5)
B: Family characteristics		
Primary caregiver	Father and mother	72 (82.8)
	Single parent	10 (11.4)
	Other	5 (5.8)
Occupation of the primary caregiver	Employed	40 (46.0)
	Never employed	47 (54.0)
Siblings living with SCA	One	50 (57.5)
	Two	30 (34.5)
	Three	7 (8.0)
Siblings who have died of SCA	One	10 (86.2)
	Two	2 (2.3)

Clinical and Haematological Outcomes of Hydroxyurea Usage by Means of Access

With regard to hydroxyurea access means (insurance, cash or projects), the median duration on hydroxyurea was 26.7 [18.9–38.7] months, 22.9 [16.5–42.2] months and 19.7 [14.6–25.4] months for insurance coverage, cash and projects, respectively.

Following hydroxyurea utilization, there was improvement of children's clinical outcomes with significant reduction in the proportions of patients reporting vaso occlusive crisis, $P < 0.0001$ in insurance group, hospital admissions ($P = 0.002$, < 0.0001 and 0.01 , in cash, insurance and Project) and blood transfusions ($P = 0.002$, < 0.0001 and < 0.0001 , in cash, insurance and Project) (Figure 2 and Table 2). Furthermore, the use of hydroxyurea was associated with significant improvement of haemoglobin (Hb) and mean corpuscular volume (MCV), as well as reduction of absolute neutrophil count (ANC) and reticulocytes among patients in the insurance and projects groups. The median Hb and MCV increased by 1.1 g/dl and 7.3 um^3 , respectively, among patients in the insurance group, and by 1.2 g/dl and 7.4 um^3 , respectively, among patients in the projects group, which was statistically significant (Table 2). Conversely, the mean ANC and percentage reticulocytes decreased by $0.8 \times 10^9/\text{L}$ and 5.7% , respectively, among patients in the insurance group, and by $1.1 \times 10^9/\text{L}$ and 6.3% , respectively, among patients in the projects group (Table 2). The changes in these parameters however did not reach statistical significance for patients who accessed hydroxyurea through cash (Table 2). The trends in Hb, MCV, ANC and reticulocytes by means of hydroxyurea access are depicted in Figure 3.

Barriers to Hydroxyurea Access

Participants faced different barriers in accessing Hydroxyurea. These barriers were exclusively reported by patients in the cash group (14% of patients) and insurance group (10% of patients; Figure 4). The reported barriers included drug unavailability in pharmacies, long distance to travel to get hydroxyurea due to unavailability in districts hospitals and also high cost of hydroxyurea that made it hard for patients in the cash group to adhere to everyday dose. For patients in the insurance group, inadequate coverage of hydroxyurea in the insurance plans and unavailability of hydroxyurea in some hospitals were the main challenges reported (Figure 4).

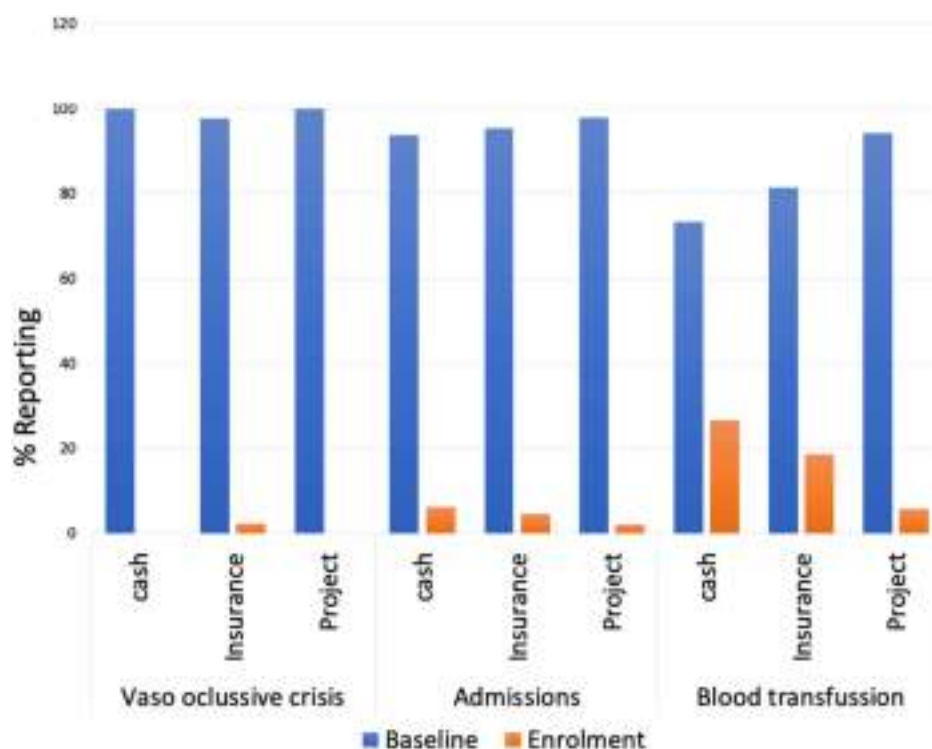


Figure 2 Reported clinical presentations at baseline and one year of Hydroxyurea.

Table 2 Haematological Outcomes of 87 Children with Sickle Cell Anaemia on Hydroxyurea

Parameter	Baseline (Before Hydroxyurea)	After Hydroxyurea Initiation (at Enrolment)	P-value
Clinical outcomes			
Vaso occlusive crisis (%)			
- Cash	16 (100.0%)	0 (0.0%)	-
- Insurance	43 (100.0%)	1 (2.3%)	< 0.0001
- Projects	28 (100.0%)	0 (0.0%)	-
Admissions (%)			
- Cash	16 (100.0%)	1 (6.3%)	0.001
- Insurance	42 (95.3%)	1 (4.6%)	<0.0001
- Projects	28 (100.0%)	2 (4.6%)	0.001
Blood transfusion (%)			
- Cash	15 (93.7%)	4 (26.7%)	0.002
- Insurance	42 (81.4%)	8 (18.6%)	<0.0001
- Projects	27 (96.4%)	1 (3.6%)	0.001
Haematological outcomes			
Haemoglobin g/dl (median [IQR])			
- Cash	7.7 [6.7–8.2]	7.8 [7.5–8.1]	0.4076
- Insurance	7.1 [5.9–8.6]	8.2 [7.4–8.9]	0.0021
- Projects	7.0 [6.2–8.4]	8.2 [7.1–9.3]	0.0081
MCV μm^3 (median [IQR])			
- Cash	79.8 [75.0–88.9]	87.7 [81.3–93.3]	0.1086
- Insurance	80.2 [74.3–84.6]	87.5 [82.7–94.9]	0.0005
- Projects	79.2 [75.9–89.2]	86.6 [80.1–94.9]	0.0162
ANC $10^9/\text{L}$ (mean$\pm$SD)			
- Cash	5.0 [3.8–8.4]	4.8 [2.6–11.1]	0.2771
- Insurance	5.3 [3.2–8.6]	4.5 [2.9–5.9]	0.0340
- Projects	5.2 [3.5–7.2]	4.1 [2.5–6.1]	0.0071
Reticulocytes % (mean$\pm$SD)			
- Cash	19.8[15.9–28.8]	9.1[6.9–13.7]	0.0732
- Insurance	18.0[14.1–20.7]	12.3[8.0–16.3]	<0.0001
- Projects	18.0[16.1–29.5]	11.7[8.2–16.3]	0.0001

Note: The bolded P values shows the clinical and haematological outcomes that improved after one year use of hydroxyurea.

Abbreviations: Hb, Haemoglobin; MCV, Mean Corpuscular Volume; ANC, Absolute Neutrophil count.

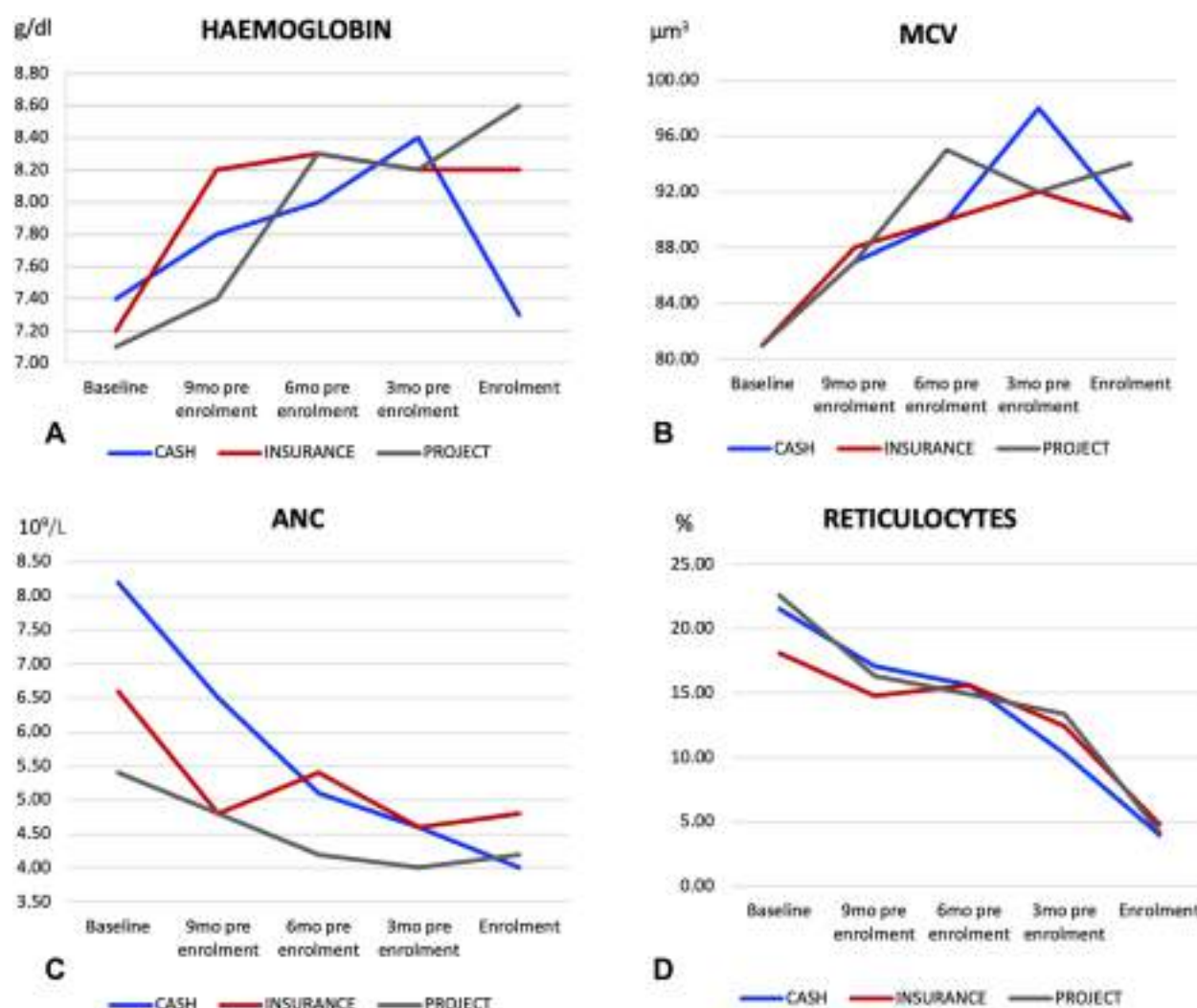


Figure 3 Showing the trend of haematological parameters ((A) Haemoglobin, (B) MCV, (C) ANC and (D) reticulocytes) to participants at baseline (before Hydroxyurea) to the time of Enrolment.

Discussion

Understanding the barriers for access to hydroxyurea once initiated is critical in ensuring sustainability of its clinical benefits. Here, we show that patients who accessed hydroxyurea via cash experienced the most challenges in getting the drug and subsequently the least haematological benefits from the drug. To the best of our knowledge, our study is one of the first to investigate the clinical and haematological benefits of hydroxyurea across various access means (cash, insurance, projects), and contributes to emerging literature on this subject. The major strength of our study is the use of two-way review (pre-hydroxyurea and post-hydroxyurea) following more than six months of use of hydroxyurea to determine its clinical and haematologic benefits in our participants.

Clinical Outcome of Hydroxyurea Usage

Our study reported significant improvement of clinical outcomes in paediatric patients with SCA on hydroxyurea, including reduction of admissions and reduction on vaso-occlusive events, which is similar to what has been reported in other studies.^{9–12} Importantly, these benefits have also been observed among children with SCA in sub-Saharan Africa. The REACH trial in sub-Saharan Africa showed clearly that the use of hydroxyurea in children had significantly reduced SCA-associated complications, blood transfusion needs and hospital admissions.¹¹ Similarly, the NORHAM study in

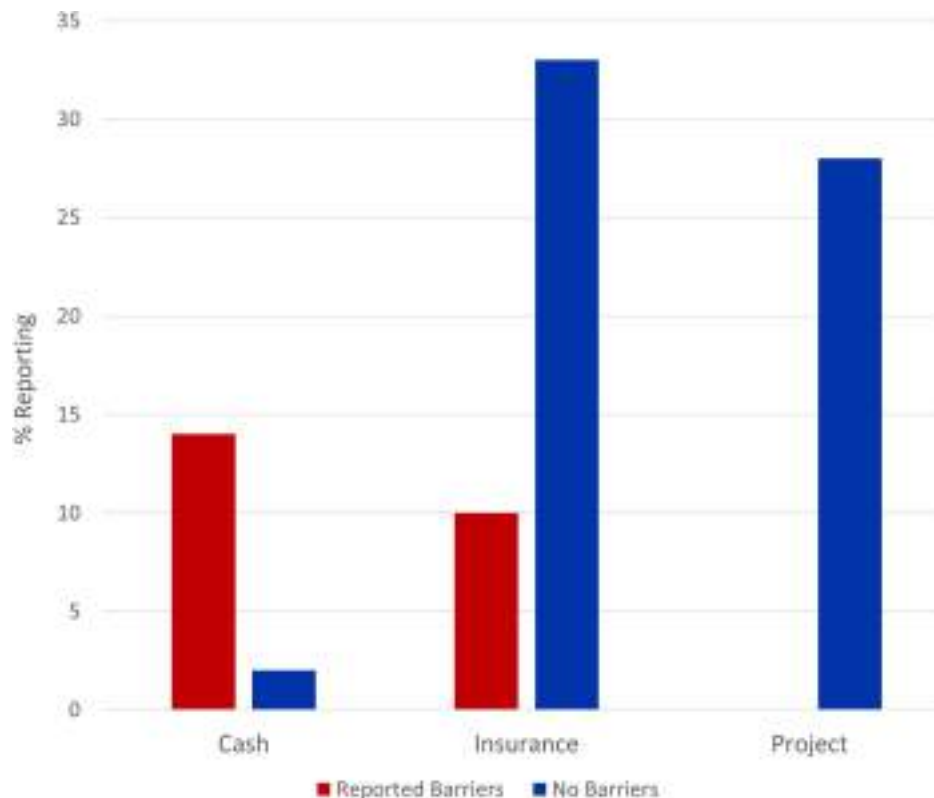


Figure 4 Barriers to Hydroxyurea access to 87 sickle cell anaemia participants on Hydroxyurea from October 2020 to April 2021.

Uganda showed decrement of vaso-occlusive crisis in children with SCA.¹² Given the mounting evidence of the clinical benefits of hydroxyurea in sub-Saharan Africa, it is high time to invest in scaling up its availability in the region with the aim to reach most if not all patients with SCA. Such roll-out will have the added advantage of facilitating cohort studies to investigate the long-term effects of hydroxyurea use in the sub-Saharan Africa settings.

Haematological Outcome of Hydroxyurea Usage by Means of Access

Dramatic improvement of laboratory values was observed in this study with increment of haemoglobin and MCV, as well as decrement of ANC and reticulocytes. Similar improvements in laboratory parameters have also been reported among children with SCA in Africa,¹⁸ including recently by the REACH study.¹¹ Nahavandi et al and Cokic et al showed that the beneficial effects of hydroxyurea in patient with SCA were mediated via induction of NO, cGMP, and HbF.^{19,20} Besides the increase in Hb and HbF, the changes in haematological parameters induced by hydroxyurea have various additional benefits to the patients. The reduction in reticulocyte count is thought to improve overall erythrocytes deformability and hence significantly reduce blood viscosity.²¹ Further, the reduction in chronic inflammation mediated by hydroxyurea also presents a major therapeutic benefit.²²

Between the three groups, we observed that those who used cash to access hydroxyurea did not experience significant changes in all the haematological parameters studied. The same group also reported inconsistent use of the drug due to financial constraints. The sub-optimal dosing of hydroxyurea that ensued likely explains the lack of haematological benefits in this group and brings to attention issues related to long-term sustainability of access to hydroxyurea, especially among the majority of patients with SCD who live in resource limited settings in sub-Saharan Africa.

Barriers to Hydroxyurea Access

Our study pointed some barriers of hydroxyurea access and the most commonly pointed out were high drug cost, insurance challenges and unavailability in most parts in the Lake Zone regions of the country where SCA is mostly

prevalent. We found that majority of the participants had to travel long distances to obtain hydroxyurea at a high cost. These challenges have also been reported by other groups.^{23–25}

In order to reduce the cost of hydroxyurea and thus increase its affordability and accessibility to patients with SCA, local production in countries that are heavily burdened with SCA may be the solution. In Africa, Nigeria has succeeded in this by having a pharmaceutical company that is producing hydroxyurea locally which has significantly reduced its costs to patients.^{26,27} In Tanzania, a proof-of-concept project at the Muhimbili University of Health and Allied Sciences (MUHAS) showed that local compounding of hydroxyurea was feasible and would reduce the cost of the drug significantly.²⁸ Such initiatives should receive full support from the industry for scale up.

Limitation

As our study was conducted during the peak of COVID-19 outbreak, we were only able to recruit a relatively small number of patients that limited our ability to examine some important factors with high resolution, particularly the barriers to hydroxyurea access across the various access means.

Conclusion

Hydroxyurea significantly increased the haemoglobin and MCV, and reduced ANC and reticulocytes in paediatric patients with SCA in Tanzania. These benefits were observed in SCA patients accessing hydroxyurea via insurance and projects, but not cash. Further, patients accessing hydroxyurea via cash reported the most challenges in accessing the drug, likely contributing to inconsistent dosing and impaired haematological benefits. While these findings support the roll-out of hydroxyurea to increased number of patients with SCA in sub-Saharan Africa, they also highlight the need to ensure its long-term affordability among patients. Future longitudinal studies should investigate the clinical and haematological outcomes as well as barriers to access of hydroxyurea across the various access means among large sample sizes of patients in North-western and Eastern parts of the country where the magnitude of SCA is highest.

Acknowledgments

We thank the patients and staff of Bugando Medical Centre. We also acknowledge support from the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number U24 HL135881 (Sickle Pan-African Research Consortium—SPARCO) and U01 HL156853 (SPARCO-Tanzania) that made this study possible. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosure

The authors declare no competing interests in this work.

References

1. Ilesanmi OO. Pathological basis of symptoms and crises in sickle cell disorder: implications for counseling and psychotherapy. *Hematol Rev*. 2010;2:10–22.
2. Prithu Sundt MT, Gladwin EMN. Pathophysiology of Sickle Cell Disease. *Physiol Behav*. 2018;176:139–148.
3. Therrell BL, Lloyd-Puryear MA, Ohene-Frempong K, et al. Empowering newborn screening programs in African countries through establishment of an international collaborative effort. *J Community Genet*. 2019;1:18.
4. Ambrose EE, Makani J, Chami N, et al. High birth prevalence of sickle cell disease in Northwestern Tanzania. *Pediatr Blood Cancer*. 2018;65:e26735. doi:10.1002/pbc.26735
5. Ambrose EE, Smart LR, Charles M, et al. Geospatial mapping of sickle cell disease in northwest Tanzania: the Tanzania sickle surveillance study (TS3). *Blood*. 2018;132:3662. doi:10.1182/blood-2018-99-113939
6. Rwezaula S, Magesa PM, Mgaya J, et al. Newborn screening for hemoglobinopathies at Muhimbili National Hospital, Dar es Salaam—Tanzania. *East Afr J Public Health*. 2015;12:948–955.
7. Saidi H, Smart LR, Kamugisha E, et al. Complications of sickle cell anaemia in children in Northwestern Tanzania. *Hematology*. 2016;21(4):248–256. doi:10.1080/10245332.2015.1101976
8. Wong TE, Brandow AM, Lim W, Lottenberg R. Update on the use of hydroxyurea therapy in sickle cell disease. *Blood*. 2014;124(26):3850–3857. doi:10.1182/blood-2014-08-435768
9. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med*. 1995;332(20):1317–1322. doi:10.1056/NEJM199505183322001

10. Cunningham-Myrie C, Abdulkadri A, Waugh A, et al. Hydroxyurea use in prevention of stroke recurrence in children with sickle cell disease in a developing country: a cost effectiveness analysis. *Pediatr Blood Cancer*. 2015;62(10):1862–1864. doi:10.1002/pbc.25563
11. Tshilolo L, Tomlinson G, Williams TN, et al. Hydroxyurea for children with sickle cell anemia in Sub-Saharan Africa. *N Engl J Med*. 2019;380(2):121–131. doi:10.1056/NEJMoa1813598
12. Opoka RO, Ndugwa CM, Latham TS, et al. Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM): a trial for children with sickle cell anemia. *Blood*. 2017. doi:10.1182/blood-2017-06-788935
13. Candrilli SD, O'Brien SH, Ware RE, et al. Hydroxyurea adherence and associated outcomes among Medicaid enrollees with sickle cell disease. *Am J Hematol*. 2011;86(3):273–277. doi:10.1002/ajh.21968
14. Qureshi A, Kaya B, Pancham S, et al. Guidelines for the use of hydroxycarbamide in children and adults with sickle cell disease: a British Society for Haematology Guideline. *Br J Haematol*. 2018;181:460–475. doi:10.1111/bjh.15235
15. Ofakunrin AOD, S Okpe E, O Afolaranmi T, et al. Level of utilization and provider-related barriers to the use of hydroxyurea in the treatment of sickle cell disease patients in jos, north-central Nigeria. *Afr Health Sci*. 2021;21:765–774. doi:10.4314/ahs.v21i2.36
16. Power-Hays A, Ware RE. Effective use of hydroxyurea for sickle cell anemia in low-resource countries. *Curr Opin Hematol*. 2020;27(3):172–180. doi:10.1097/moh.0000000000000582
17. Kilonzi M, Mlyuka HJ, Felician FF, et al. Barriers and Facilitators of Use of Hydroxyurea among Children with Sickle Cell Disease: experiences of Stakeholders in Tanzania. *Hemato*. 2021;2(4):713–726. doi:10.3390/hemato2040048
18. Ware RE. Optimizing hydroxyurea therapy for sickle cell anemia. *Hematol Am Soc Hematol Educ Progr*. 2015;2015:436–443. doi:10.1182/asheducation.V2015.1.436.3917688
19. Nahavandi M, Tavakkoli F, Wyche MQ, et al. Nitric oxide and cyclic GMP levels in sickle cell patients receiving hydroxyurea. *Br J Haematol*. 2002;119(3):855–857. doi:10.1046/j.1365-2141.2002.03919.x
20. Cokic VP, Andric SA, Stojilkovic SS, Noguchi CT, Schechter AN. Hydroxyurea nitrosylates and activates soluble guanylyl cyclase in human erythroid cells. *Blood*. 2008;111:1117. doi:10.1182/blood-2007-05-088732
21. Davies SC, Gilmore A. The role of hydroxyurea in the management of sickle cell disease. *Blood Rev*. 2003;17:99–109. doi:10.1016/S0268-960X(02)00074-7
22. Conran N, Belcher JD. Inflammation in sickle cell disease. *Clin Hemorheol Microcirculat*. 2018. doi:10.3233/CH-189012
23. Badawy SM, Thompson AA, Penedo FJ, et al. Barriers to hydroxyurea adherence and health-related quality of life in adolescents and young adults with sickle cell disease. *Eur J Haematol*. 2017;98:608–614. doi:10.1111/ejh.12878
24. Adeyemo TA, Diaku-Akinwunmi IN, Ojewunmi OO, Bolarinwa AB, Adekile AD. Barriers to the use of hydroxyurea in the management of sickle cell disease in Nigeria. *Hemoglobin*. 2019;43:188–192. doi:10.1080/03630269.2019.1649278
25. Okocha EC, Gyamfi J, Ryan N, et al. Barriers to therapeutic use of hydroxyurea for sickle cell disease in Nigeria: a cross-sectional survey. *Front Genet*. 2022;12:2834. doi:10.3389/fgene.2021.765958
26. Abdullahi SU, Jibir BW, Bello-Manga H, et al. Hydroxyurea for primary stroke prevention in children with sickle cell anaemia in Nigeria (SPRING): a double-blind, multicentre, randomised, Phase 3 trial. *Lancet Haematol*. 2022;9:e26–e37. doi:10.1016/S2352-3026(21)00368-9
27. Galadanci NA, Abdullahi SU, Ali Abubakar S, et al. Moderate fixed-dose hydroxyurea for primary prevention of strokes in Nigerian children with sickle cell disease: final results of the SPIN trial. *Am J Hematol*. 2020;95:E247. doi:10.1002/ajh.25900
28. Costa E, Tibalinda P, Sterzi E, et al. Making hydroxyurea affordable for sickle cell disease in Tanzania is essential (HASTE): how to meet major health needs at a reasonable cost. *Am J Hematol*. 2021;96:E2–E5. doi:10.1002/ajh.26007

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Successful CNS-Centric Therapeutic Management and Genomic Profiling of Primary Cranial Vault Diffuse Large B-Cell Lymphoma

Jordan Davis, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Liuyan Jiang, Vivek Gupta, Ephraim Parent & Han W Tun

To cite this article: Jordan Davis, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Liuyan Jiang, Vivek Gupta, Ephraim Parent & Han W Tun (2023) Successful CNS-Centric Therapeutic Management and Genomic Profiling of Primary Cranial Vault Diffuse Large B-Cell Lymphoma, Journal of Blood Medicine, , 49-55, DOI: [10.2147/JBM.S391094](https://doi.org/10.2147/JBM.S391094)

To link to this article: <https://doi.org/10.2147/JBM.S391094>



© 2023 Davis et al.



Published online: 22 Jan 2023.



Submit your article to this journal



Article views: 260



View related articles



View Crossmark data

Successful CNS-Centric Therapeutic Management and Genomic Profiling of Primary Cranial Vault Diffuse Large B-Cell Lymphoma

Jordan Davis¹, ErinMarie O Kimbrough¹, Muhamad Alhaj Moustafa¹, Liuyan Jiang², Vivek Gupta³, Ephraim Parent³, Han W Tun¹

¹Division of Hematology and Oncology, Mayo Clinic, Jacksonville, FL, USA; ²Department of Pathology, Mayo Clinic, Jacksonville, FL, USA; ³Department of Radiology, Mayo Clinic, Jacksonville, FL, USA

Correspondence: Han W Tun, Division of Hematology and Oncology, Mayo Clinic, 4500 San Pablo Road South, Jacksonville, FL, 32224, USA, Tel +1 904 953 7290, Fax +1 904 953 2315, Email Tun.Han@mayo.edu

Abstract: Primary cranial vault lymphoma (PCVL) is a rare lymphoma involving the skull with or without extra- and intracranial extension. Most cases of PCVL are diffuse large B-cell lymphoma (DLBCL). We report a case of primary cranial vault diffuse large B-cell lymphoma (PCV-DLBCL) that was successfully treated with anthracycline-based chemoimmunotherapy (CIT) alternating with central nervous system (CNS)-directed CIT with high-dose methotrexate and high-dose cytarabine. CNS-centric therapy was given for suspected cerebral cortical involvement and presumed elevated risk of CNS recurrence. The patient has remained in complete remission for 4.25 years following treatment. We suggest that PCV-DLBCL is potentially curable with CNS-directed therapy. Additionally, we provide genomic profiling results indicating an indeterminate cell of origin and multiple genetic mutations which are not frequently seen in DLBCL.

Keywords: diffuse large B-cell lymphoma, central nervous system, calvarium, chemotherapy, scalp lesions

Introduction

The World Health Organization (WHO) recognizes diffuse large B-cell lymphomas (DLBCL) localized to specific extranodal sites.¹ These extranodal lymphomas include primary central nervous system (CNS) lymphoma, primary vitreo-retinal lymphoma, primary testicular lymphoma, primary cutaneous large B-cell lymphoma, and primary effusion lymphoma.¹

Primary cranial vault lymphoma (PCVL), or calvarial lymphomas, are extranodal non-Hodgkin lymphomas characterized by localized involvement of the cranial vault with tri-compartment (extracranial, calvarial, and intracranial) involvement.⁴ PCVL is rare and has been sparsely reported in the medical literature.²⁻⁴ Patients often present with painless scalp lesions with normal overlying skin. Neurologic symptoms vary by location and extent of disease.^{3,4,9} The diagnosis is established with a combination of imaging and tissue biopsy of the identified mass.^{3,9} PCVL is managed with surgical resection of the tumor, radiation therapy, systemic chemotherapy, or a combination of these therapies.^{4,6,9,12} The optimal treatment approach remains uncertain, however, given the rarity of this entity and limited reports of long-term follow-up.⁴ We present a case of PCVL successfully treated with CNS-centric systemic chemoimmunotherapy (CIT).

Case Presentation

A 69-year-old Caucasian male with a history of chronic headaches presented with rapidly enlarging scalp masses involving the left periauricular and temporal areas that varied in size and shape and were sensitive to touch. He also noted worsening frontal headaches, dizziness, and cramping of the jaw. Seven months prior, these scalp lesions were presumed to be shingles, and he was treated with valacyclovir without improvement in his symptoms. Initial magnetic

resonance imaging (MRI) and computed tomography (CT) scans of the head revealed an incidental pituitary microadenoma but did not explain his scalp lesions.

Given the worsening symptoms, repeat imaging was obtained. MRI and positron emission tomography–computed tomography (PET-CT) demonstrated a 1.8×3.5 cm subgaleal hypermetabolic tumor of the scalp in the parietal area with permeative erosion of the adjacent calvarial bone and intracranial extension with extensive pachymeningeal involvement (Figure 1A–C). In addition, hypermetabolic changes were seen in three regional lymph nodes in the left preauricular region (Figure 2A). The tumor was resected, and pathology revealed a diffuse proliferation of large, atypical lymphocytes in a fibrotic background adjacent to periosteal soft tissue and attached to the dura (Figure 3A and inset). The neoplastic lymphocytes were diffusely positive for CD20, CD10, BCL2, and BCL6 by immunohistochemistry, and 90% of cells stained positive for Ki-67 (Figure 3B–3F). The lymphocytes were negative for MUM1, MYC, CD30, and CD23. Myeloperoxidase, CD1a, S100, and epithelial membrane antigen (EMA) were also negative. Epstein-Barr encoded RNA (EBER) in situ hybridization (ISH) was negative. Fluorescence in situ hybridization (FISH) studies were negative for MYC/IGH translocation. The tissue was also sent for comprehensive genomic analysis with GTC-Hematology Profile Plus by Genomic Testing Cooperative which combines expression and fusion with mutation analysis in DNA and RNA. The test covers 179 DNA genes and 1408 RNA genes which are known to be associated with hematologic malignancies. The genomic analysis supported the diagnosis of DLBCL. These results are summarized in Table 1. Based on Han's criteria, the cell of origin (COO) was initially classified as germinal center B-cell (GCB) subtype of DLBCL; however, the genomic findings including gene expression signature indicated mixed COO.

He was diagnosed with stage IV primary cranial vault diffuse large B-cell lymphoma (PCV-DLBCL) with an International Prognostic Index score of 3 based on age >60, stage IV disease, and involvement of 2 extranodal sites. Initial laboratory investigations including complete blood count, comprehensive metabolic profile, and lactate dehydrogenase were all within normal limits. Lumbar puncture was deferred given the concern for CNS involvement and plan for CNS-directed therapy. He was treated with rituximab 375 mg/m², cyclophosphamide 750 mg/m², doxorubicin 50 mg/m², and vincristine 2 mg on day 1, and prednisone 100 mg daily on days 1–5 (R-CHOP), alternating with rituximab (R) 375 mg/m², high-dose (HD)-methotrexate (MTX) 3.5 g/m² on day 1, and high-dose cytarabine (HiDAC) 1000 mg/m² q12h on days 2–3 (R-MA regimen) for a total of eight cycles. HiDAC was reduced by 20% with cycle six due to pancytopenia requiring hospitalization. It was further reduced by 20% for the final cycle due to cytopenias. He achieved a complete remission (CR) after four cycles of CIT and has remained in CR for 4.25 years with no adverse sequelae from his lymphoma or lymphoma treatment (Figure 2A–H).

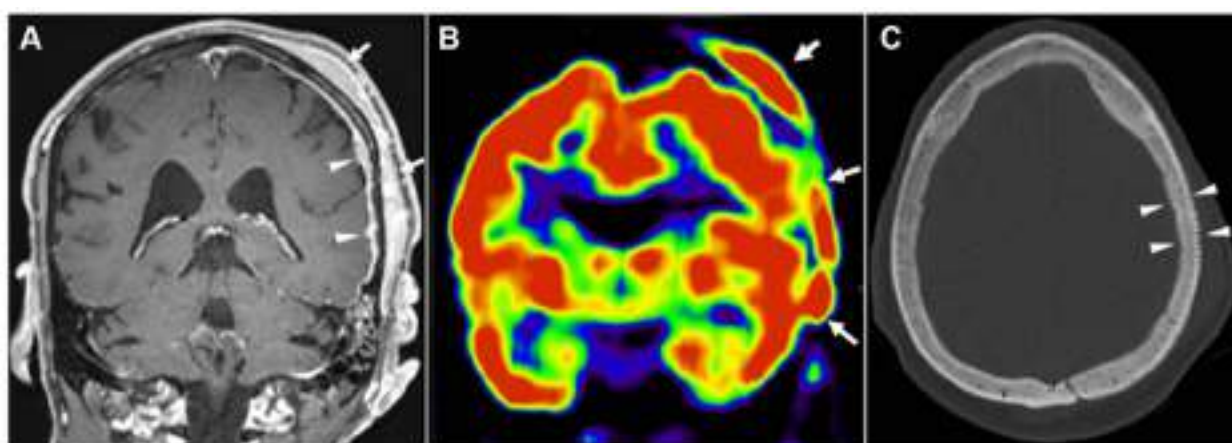


Figure 1 Initial FDG PET-MRI Images. (A) Coronal contrast enhanced T1 weighted MRI shows infiltrating subgaleal mass (arrows) with abnormal enhancement of the underlying calvarial marrow and nodular thickening of the dura (arrowheads). (B) The infiltrating mass is hypermetabolic on FDG-PET (arrows). (C) High-resolution CT of the skull reveals permeative erosions and slightly increased density of the involved parietal bone.

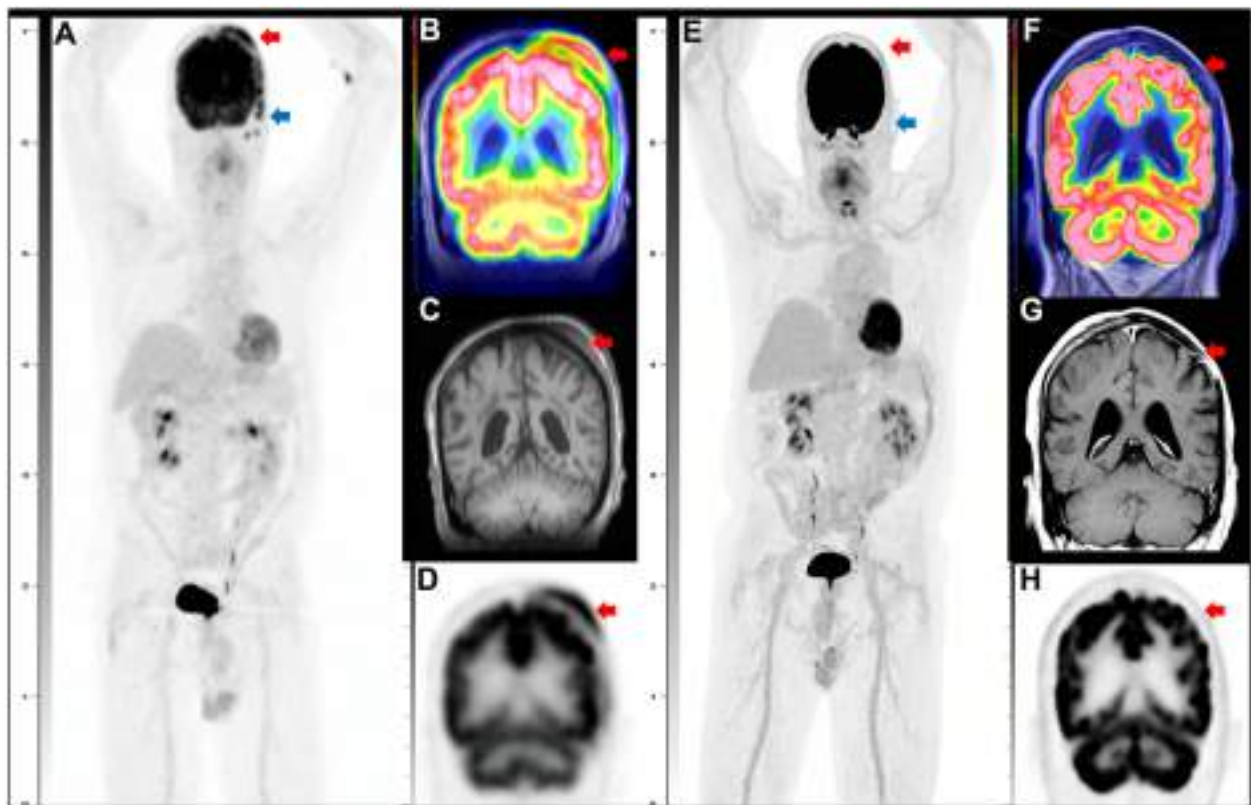


Figure 2 Initial and Post-Treatment FDG PET-MRI. Composite fused FDG PET-MRI images at initial diagnosis and after treatment with R-CHOP alternating with R-MA. Coronal view of FDG PET maximum intensity projection (MIP) at initial diagnosis (**A**) demonstrates moderately hypermetabolic soft tissue in the scalp subcutaneous tissues with SUV max of 7.8 (red arrow). The subcutaneous tissue has transcalvarial extension and involvement of the underlying pachymeninges as seen on fused coronal FDG PET-MRI (**B**), T1 MRI (**C**), and FDG PET (**D**) (red arrows). Initial FDG PET MIP (**A**) also demonstrates hypermetabolic left preauricular lymph nodes and temporalis muscle involvement (blue arrow). Post-treatment fused FDG PET-MRI MIP (**E**) demonstrates complete resolution of the scalp subcutaneous tissues (red arrows) and lymphadenopathy (blue arrow) and is confirmed on coronal fused PET-MRI (**F**), T1 + contrast (**G**) and FDG PET (**H**) (red arrows).

Discussion

PCVL is a rare extranodal lymphoma that involves the skull with or without extra- and intracranial extension (ie, the scalp, calvarium, and intracranial space).^{3,4} It accounts for 0.2% of all lymphomas, and PCV-DLBCL represents 0.3–0.5% of extranodal non-Hodgkin lymphomas.^{2,14} PCVL appears to be quite distinct from primary dural lymphoma, which is predominantly an extranodal marginal zone B-cell lymphoma.¹¹ PCVL is more common among Caucasian individuals in their 7th decade of life.³ Patients present with painless scalp lesions with normal overlying skin and often endorse regional headache.^{3,4,9} Neurologic symptoms including seizures, mental status changes, apraxia, and hemiparesis may be present as a result of intracranial extension or mass effect.⁴ PCVL should be considered in a patient presenting with a scalp mass with or without headache or other neurologic symptoms.^{3,9}

A diagnosis of PCVL can be made after evaluation with imaging (MRI, CT, or PET-CT) and biopsy of the affected area.^{3,9} On imaging, PCVL may involve all three compartments of the cranial vault (scalp, calvarium, and dura). Tri-compartment involvement was reported in 87% of patients with PCVL in one meta-analysis.^{3,9} Bone destruction and parenchymal brain involvement may also be seen on imaging but are more common in advanced disease.¹⁰ Early in the disease course, these cancers cause little to no bone destruction despite transcalvarial involvement. In later stages of disease, however, there is often extensive infiltrative destruction of the skull.^{2–7} The lymphoma cells infiltrate the diploic spaces and extend along emissary veins to involve the soft tissue on both sides of the skull, resulting in enlargement of the subcutaneous scalp mass without initial damage to the intervening bone.^{3–5,8} This infiltrative pattern of bone destruction is unique to PCVL.⁵ Patients with complete bone destruction without brain parenchymal disease, as well as those with parenchymal involvement without bone destruction have been reported.^{2,4,10} Involvement of the brain parenchyma should be suspected if

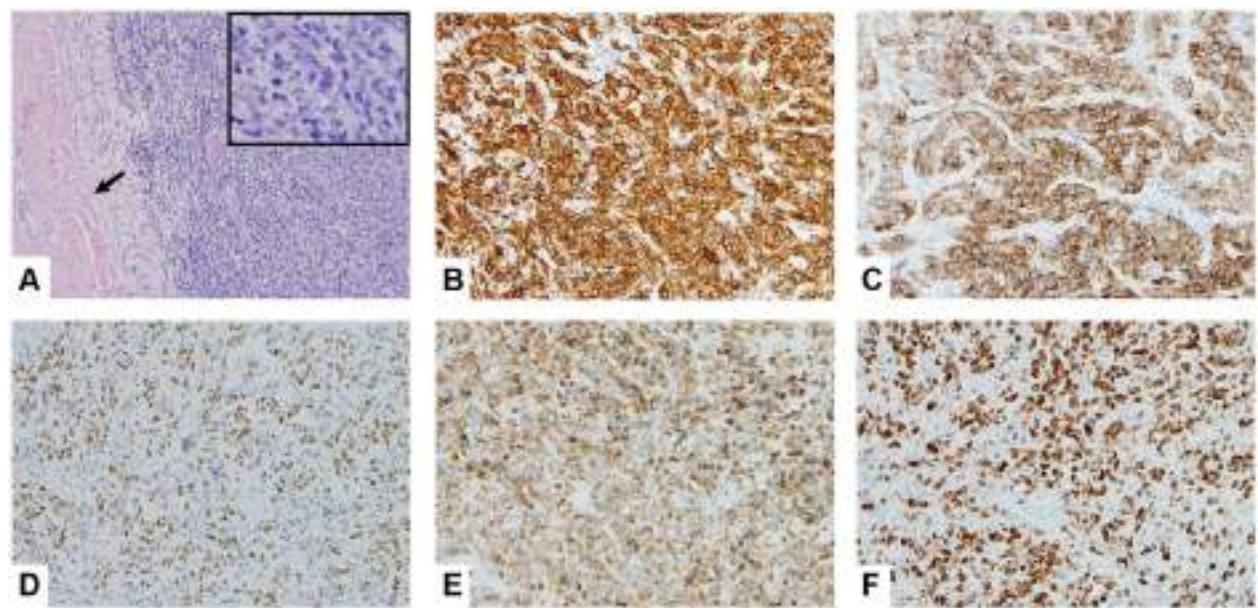


Figure 3 Pathologic Evaluation of Resected Tumor. The H&E sections (**A**, x 10) show the proliferation of neoplastic lymphocytes adjacent to the periosteal soft tissue (arrow); and neoplastic lymphocytes are large with open chromatin and prominent nucleoli; mitosis is brisk (inset, x 40). They are positive for CD20, CD10, BCL6, BCL2, with a high proliferative rate by Ki-67, 90% (**B–F**, x 20).

the MRI demonstrates an indistinguishable border between the meninges and cortex in the presence of subcortical edema.³ In our patient, the tumor exhibited involvement of the three compartments with the parietal bone showing minimal osteolytic changes, extracranial extension into the scalp with sub-galeal tumor, and intracranial extension with extensive hemispheric pachymeningeal involvement in association with three regional lymph nodes without evidence of distant disease. Patients with PCVL may also develop distant disease due to hematogenous spread, and PET-CT can help identify metastasis.⁹

Table I Pathologic and Genomic Findings in a Patient with PCV-DLBCL

Pathology Findings	
Immunohistochemistry	Positive - CD20, CD10, BCL2, BCL6
	Negative - MUM1, MYC, CD30, CD23
EBER ISH	Negative
Cell of origin (Han's criteria)	Germinal center B cell subtype
Genomic Profile ^a	
Mutation Profile	KRAS 183A>C, EZH2 1936T>A, MYD88 814C>T, XPO1 1654C>T, STAT3 2147C>T, ATRX 2975–2982del, TNFAIP3 617C>T and 547C>T, KMT2B 6137delG, HGF 1505G>A, POT1 1632G>A, CD22 381A>T, EBF1 416T>G, CD58 168G>A, NIPBL 5044C>T
Chromosomal Analysis	6p+, 10p+, +19 No translocation involving BCL2, BCL6, and MYC
Cell of Origin (Gene Expression Profiling)	Indeterminate Cell of origin

Note: ^aBased on GTC Hematology Profile Plus by Genomic Testing Cooperative.
Abbreviations: ABC, activated B-cell; CIT, chemoimmunotherapy; CNS, central nervous system; COO, cell of origin; CR, complete remission; CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; EBER, Epstein-Barr encoded RNA; EMA, epithelial membrane antigen; FISH, fluorescence in situ hybridization; GCB, germinal center B-cell; HD, high-dose; HiDAC, high-dose cytarabine; ISH, in situ hybridization; MRI, magnetic resonance imaging; MTX, methotrexate; OS, overall survival; PET-CT, positron emission tomography-computed tomography; PCV-DLBCL, primary cranial vault-diffuse large B-cell lymphoma; PCVL, primary cranial vault lymphoma; R, rituximab; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; WHO, world health organization.

A recently published meta-analysis evaluating 62 patients with PCVL revealed that the majority of cases result from B-cell lymphomas (58/62 cases) with only 1 reported case of T-cell lymphoma.⁹ The majority were aggressive B-cell lymphomas [DLBCL (61%), Burkitt lymphoma (5%), and high-grade B-cell lymphoma (3%)], however, low-grade B-cell lymphomas were also reported (follicular lymphoma, extranodal marginal zone B-cell lymphoma, small lymphocytic lymphoma, and unspecified low-grade B-cell lymphomas).⁹ Genomic data of PCVL are lacking.

Our report appears to be the first one to provide comprehensive genomic profiling results on PCV-DLBCL. The genetic profile of the case is quite interesting in that the cell of origin (COO) and molecular subtype cannot be definitively classified. The algorithm used by GTC Hematology Plus indicates that the COO is unclassifiable/mixed based on the gene expression profile. Out of fifteen mutations detected, EZH2 mutation is frequently associated with GCB COO whereas TNFAIP3 and MYD88 mutations are frequently seen in non-GCB COO.^{17,18} The remaining mutations are not frequently found in DLBCL. The MYD88 814C>T mutation found in our patient is a truncating mutation and has been associated with innate immune deficiency.¹⁹ It is distinct from MYD88 L265P which is frequently associated with non-GCB DLBCL¹⁷ and is the most common mutation in primary CNS lymphoma.²⁰ As for molecular subtype, EZH2 mutation suggests EZB subtype.^{25,26} However, TNFAIP3 and STAT3 mutations are not typically seen in EZB subtype. TNFAIP3 mutation is associated with BN2 whereas STAT3 mutation is associated with ST2.^{25,26} Based on mutation profile, multiple signaling pathways are affected: epigenetic regulation (EZH2, ATRX, and KMT2B), JAK-STAT signaling (STAT3), NFκB signaling (TNFAIP3 and MYD88), B cell related pathways (CD22, EBF1, and CD58), chromosome regulation (POT1 and NIPBL), cell proliferation (KRAS and HGF), and regulation of nuclear export (XPO1).^{27,28}

The optimal therapeutic approach for PCVL is not well characterized given its rarity.⁴ Historically, surgical resection of the tumor, radiation therapy, and systemic chemotherapy with CHOP have been used.^{4,6,9,12} Treatment with surgical excision alone is likely insufficient and may not improve remission rates when compared to systemic therapy.^{9,12} There are several reports of patients with PCV-DLBCL without CNS involvement achieving CR with radiotherapy alone, or in combination with systemic chemotherapy without CNS-penetrating agents.^{5,9,21–23} The longest reported follow-up was 4.8 years.²¹ Two other patients achieved CR with surgery and radiotherapy alone, but these patients had mucosa-associated lymphoid tissue (MALT) lymphoma, a subtype of non-Hodgkin lymphoma known to be particularly sensitive to radiotherapy that is distinct from DLBCL.^{4,10} CNS-penetrating chemotherapeutics have been used previously to treat PCV-DLBCL.^{5,13} One patient received rituximab in combination with cytarabine following surgical resection, while the other received methotrexate alone, followed by rituximab, cyclophosphamide, cytarabine, etoposide, and dexamethasone.^{5,13} Both patients achieved a CR.

R-CHOP is the standard treatment for systemic DLBCL, but it is ineffective for treatment of CNS lymphoma due to its poor CNS penetration.¹⁶ We utilized a CNS-centric therapeutic approach for our patient given the concern for cerebral involvement. Our patient received a combination of R-CHOP alternating with Rituximab, HiDAC, and HD-MTX. He achieved CR after four cycles of CIT and has remained in CR for 4.25 years.

There are limited overall survival (OS) data for PCVL given the scarcity of reported cases in the literature and lack of long-term follow up. In one small case series, the median OS was reported to be 17.8 months in patients without cerebral cortical involvement and 10.5 months for those with PCVL involving the brain parenchyma.¹³ Ultimately, given the risk of cerebral involvement in patients with PCVL, we believe CNS-directed therapies should be considered in its management, particularly PCV-DLBCL and other high-grade B-cell lymphoma subtypes.^{9,15}

Extensive genomic profiling of lymphomas is becoming more common; however, it is still not widely employed and the utility of genomic testing to predict response to therapy is limited.²⁴ With further genomic characterization of these tumors, we may be better equipped to individualize treatment to improve outcomes.

Conclusion

PCV-DLBCL is a rare primary extranodal lymphoma of the calvarium with or without extracranial and intracranial extension. The genomic profiling of our case revealed an indeterminate COO and multiple genetic mutations which are not commonly seen in DLBCL. While there is no standard treatment for PCV-DLBCL, we suggest that a CNS-centric therapeutic approach is warranted due to high CNS risk and is potentially curative. More research is needed to have a better understanding of the biology of PCV-DLBCL and identify an optimal therapeutic approach.

Consent for Publication

The study participant has given written informed consent to participate as well as consent to publish their data and images. IRB approval was not required to publish the case details.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leukemia*. 2022;36(7):1720–1748. doi:10.1038/s41375-022-01620-2
2. Kanaya M, Endo T, Hashimoto D, et al. Diffuse large B-cell lymphoma with a bulky mass in the cranial vault. *Int J Hematol*. 2017;106(2):147–148. doi:10.1007/s12185-017-2229-x
3. da Rocha AJ, da Rocha TM, da Silva CJ, Paes RP, Bruniera P, Chiattoni CS. Cranial vault lymphoma: a systematic review of five patients. *J Neurooncol*. 2010;100(1):9–15. doi:10.1007/s11060-010-0137-5
4. Kosugi S, Kume M, Sato J, et al. Diffuse large B-cell lymphoma with mass lesions of skull vault and ileocecum. *J Clin Exp Hematop*. 2013;53(3):215–219. doi:10.3960/jslrt.53.215
5. Umemura T, Nakano Y, Soejima Y, et al. Characteristics of bone destruction in cranial vault lymphoma compared with other skull tumors. *J UOEH*. 2019;41(3):335–342. doi:10.7888/juoeh.41.335
6. Jaiswal M, Gandhi A, Purohit D, Singhvi S, Mittal RS. Primary non-Hodgkin's lymphoma of the skull with extra and intracranial extension presenting with bulky scalp mass lesion. *Asian J Neurosurg*. 2016;11(4):444. doi:10.4103/1793-5482.145093
7. Lee SH, Yun SJ. Early-stage primary cranial vault lymphoma in a 50-year-old man: presenting as only sclerosis and mimicking osteoma. *Ann Hematol*. 2018;97(1):183–184. doi:10.1007/s00277-017-3121-8
8. Mongia S, Shukla D, Devi BI, Reddy TV. Primary cranial vault non-Hodgkin's lymphoma. *Neurol India*. 2003;51(2):293–294.
9. Toyota E, Taslimi S, Alkins R. Optimal management of calvarial lymphoma: a meta-analysis. *World Neurosurg*. 2021;151:e484–e494. doi:10.1016/j.wneu.2021.04.066
10. El Asri AC, Akhaddar A, Baallal H, et al. Primary lymphoma of the cranial vault: case report and a systematic review of the literature. *Acta Neurochir*. 2012;154(2):257–265. doi:10.1007/s00701-011-1124-0
11. Dobran M, Paracino R, Mancini F, Nasi D. Primary dural lymphoma: clinical cases and literature review. *Case Rep Med*. 2020;21(2):2812487.
12. Chan DYC, Chan DTM, Poon WS, Wong GKC. Primary cranial vault lymphoma. *Br J Neurosurg*. 2018;32(2):214–215. doi:10.1080/02688697.2016.1226262
13. Ciarpaglini R, Otten P. Primary cranial vault lymphoma with brain infiltration: case report and review of the literature. *Br J Neurosurg*. 2012;26(5):756–758. doi:10.3109/02688697.2012.665515
14. Uchida T, Amagasaki K, Hosono A, et al. Primary diffuse large B-cell lymphoma of the cranial vault with Trousseau syndrome: a case report. *J Med Case Reports*. 2021;15:431. doi:10.1186/s13256-021-02979-z
15. Bernstein SH, Unger JM, Leblanc M, Friedberg J, Miller TP, Fisher RI. Natural history of CNS relapse in patients with aggressive non-Hodgkin's lymphoma: a 20-year follow-up analysis of SWOG 8516 – the Southwest Oncology Group. *J Clin Oncol*. 2009;27(1):114–119. doi:10.1200/JCO.2008.16.8021
16. Tai WM, Chung J, Tang PL, et al. Central nervous system (CNS) relapse in diffuse large B cell lymphoma (DLBCL): pre- and post-rituximab. *Ann Hematol*. 2011;90(7):809–818. doi:10.1007/s00277-010-1150-7
17. Chapuy B, Stewart C, Andrew J, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med*. 2018;24:679–690. doi:10.1038/s41591-018-0016-8
18. Honma K, Tsuzuki S, Nakagawa M, et al. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. *Blood*. 2009;114:2467–2475. doi:10.1182/blood-2008-12-194852
19. Platta CD, Zamana F, Jacqueline G, et al. A novel truncating mutation in MYD88 in a patient with BCG adenitis, neutropenia and delayed umbilical cord separation. *Clin Immunol*. 2019;207:40–42. doi:10.1016/j.clim.2019.07.004
20. Bjoern Chapuy MG, Roemer M, Stewart C, et al. Targetable genetic features of primary testicular and primary central nervous system. lymphomas. *Blood*. 2016;127(7):869–881. doi:10.1182/blood-2015-10-673236
21. Yoshida K, Kagami H, Shimazaki K, et al. Malignant lymphoma of the cranial vault: analysis of three cases. *Neuropathology*. 1999;19:283–287. doi:10.1046/j.1440-1789.1999.00246.x
22. Naama O, Gazzaz M, Boucetta B, Elmoustarchid B. Elmoustarchid. Primary non-Hodgkin lymphoma of the cranial vault. *Rev Neurol*. 2017;173(10):668–670. doi:10.1016/j.neurol.2017.05.011
23. Senapati SB, Mishra SS, Dhir MK, Das S, Burma S. Primary skull lymphoma: a case report and review of similar cases. *South Asian J Cancer*. 2013;2(4):287. PMID: 24455662; PMCID: PMC3889065. doi:10.4103/2278-330X.119908
24. de Leval L, Alizadeh A, Bergsagel P, et al. Genomic profiling for clinical decision making in lymphoid neoplasms. *Blood*. 2022;140(21):2193–2227. doi:10.1182/blood.2022015854
25. Sehn L, Salles G. Diffuse large B cell lymphoma. *N Engl J Med*. 2021;384(9):842–858. doi:10.1056/NEJMra2027612
26. Weber T, Schmitz R. Molecular subgroups of diffuse large B cell lymphoma: biology and implications for clinical practice. *Curr Oncol Rep*. 2022;24:13–21. doi:10.1007/s11912-021-01155-2
27. Stelzer G, Rosen R, Plachkes I, et al. The genecards suite: from gene data mining to disease genome sequence analysis. *Curr Protoc Bioinformatics*. 2016;54(1):1–30. doi:10.1002/cpbi.5
28. Safran M, Rosen N, Twik M, et al. The genecards suite chapter. In: *Practical Guide to Life Science Databases*. Springer; 2022:27–56.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Association Between *Helicobacter pylori* Infection and Anemia Among Adult Dyspeptic Patients Attending Kiryandongo General Hospital, Uganda

Daisy Asiimwe, Isaac Bangi, Jospeh Esanyu, Daniel Ojok, Benedict Okot, Clinton Olong, Robert Wagubi, Godfrey Kisembo, Fred Sempijja, Enoch Muwanguzi & Benson Okongo

To cite this article: Daisy Asiimwe, Isaac Bangi, Jospeh Esanyu, Daniel Ojok, Benedict Okot, Clinton Olong, Robert Wagubi, Godfrey Kisembo, Fred Sempijja, Enoch Muwanguzi & Benson Okongo (2023) Association Between *Helicobacter pylori* Infection and Anemia Among Adult Dyspeptic Patients Attending Kiryandongo General Hospital, Uganda, Journal of Blood Medicine, , 57-66, DOI: [10.2147/JBM.S392146](https://doi.org/10.2147/JBM.S392146)

To link to this article: <https://doi.org/10.2147/JBM.S392146>



© 2023 Asiimwe et al.



Published online: 24 Jan 2023.



Submit your article to this journal



Article views: 824



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Association Between *Helicobacter pylori* Infection and Anemia Among Adult Dyspeptic Patients Attending Kiryandongo General Hospital, Uganda

Daisy Asiimwe¹, Isaac Bangi¹, Josph Esanyu¹, Daniel Ojok¹, Benedict Okot¹, Clinton Olong¹, Robert Wagubi¹, Godfrey Kisembo², Fred Sempijja³, Enoch Muwanguzi¹, Benson Okongo¹

¹Department of Medical Laboratory Science, Mbarara University of Science and Technology, Mbarara City, Uganda; ²Department of Hospital Administration, Kiryandongo General Hospital, Kiryandongo, Uganda; ³Department of Medical Laboratory Technology, Uganda Institute of Allied Health and Management Sciences - Mulago, Kampala City, Uganda

Correspondence: Benson Okongo, Department of Medical Laboratory Science, Mbarara University of Science and Technology, P.O. BOX 1410, Mbarara City, Uganda, Tel +256 778 557 867, Fax +256-485-20782, Email bokongo@must.ac.ug

Purpose: To determine the prevalence of anemia and its association with *Helicobacter pylori* infection among adult dyspeptic patients.

Patients and Methods: A cross-sectional study was conducted among 283 dyspeptic patients at Kiryandongo General Hospital, in Uganda. A structured questionnaire was administered to capture demographic and clinical characteristics of study participants. Four milliliters of blood were then collected into an EDTA vacutainer for Complete Blood Count (CBC) and analyzed using HUMA COUNT 30^{TS}, and peripheral blood smears were made and stained using Giemsa stain. Anemia was defined as hemoglobin levels <12g/dl in females and <13g/dl in men according to the World Health Organization (WHO). *Helicobacter pylori* (*H. pylori*) stool antigen test was performed using Whole power *H. pylori* Ag rapid test device, and saline stool preparation was examined for intestinal parasites. Chi-squared test and Logistic regression were performed to determine association, and a p-value of ≤0.05 was considered statistically significant.

Results: The overall prevalence of *Helicobacter pylori* infection was 42.4% (120/283). The prevalence of anemia among *H. pylori*-infected patients was 25.8% (31/120) and 15.3% (25/163) among *H. pylori*-negative counterparts. *H. pylori* infection was significantly associated with anemia (p-value 0.042), age (p-value 0.02, 0.009), water sources (p-value 0.0049,) and intestinal parasitic infestation (p-value 0.02), respectively.

Conclusion: This study has shown that the prevalence of *H. pylori* infection and anemia is high among dyspeptic patients at Kiryandongo General Hospital. *H. pylori* infection was found associated with anemia, age, water sources, and intestinal parasitic infestation. Routine screening of anemia in *H. pylori*-infected individuals and further studies to explore the relationship between anemia and *H. pylori* disease is highly recommended.

Keywords: anemia, prevalence, *Helicobacter pylori* infection

Introduction

Helicobacter pylori (*H. pylori*) infection is a global public health problem affecting both developed and developing countries^{1,2} with a higher burden of 50.8% reported in developing countries compared to 34.7% in developed countries.³ *Helicobacter pylori* is a helix-shaped, curved rod and gram-negative bacteria. It causes gastritis, peptic ulcer disease, gastroduodenal ulcer, atrophic gastritis, gastric cancers, and dyspeptic symptom.^{4,5} However, more than 80% of persons who become infected with *H. pylori* are usually asymptomatic. *H. pylori* plays a vital role in the natural stomach ecology.⁶

H. pylori infection affects about 4.4 billion people worldwide.¹ Africa has the highest prevalence of the infection, 70.1%, and the prevalence ranged from 18.9% in Switzerland to 87.7% in Nigeria.¹ In Southern Asia, Pakistan and India

showed the highest prevalence and in Western Asia, Turkey reported the leading prevalence, 77.2%.⁶ In Uganda, the prevalence of *H. pylori* among dyspeptic patients was 74%.⁷

H. pylori infection has been implicated in hematological manifestations such as anemia and micronutrient deficiency (iron and vitamin B12).⁸ It has also been related to extra-gastric manifestations, eg thrombocytopenic purpura, reduction in growth velocity, iron deficiency, and/or anemia.^{9,10} Several studies described that by eliminating *H. pylori* bacteria, the iron nutritional status becomes normal without the necessity for iron supplementation.^{11–14}

The mechanism or mechanisms through which *H. pylori* infection may cause iron deficiency and/or anemia are not fully understood but probable mechanisms comprise an upsurge in intragastric pH; reduced concentration of ascorbic acid in gastric juices, which disturbs iron absorption from the diet; chronic bleeding caused by the increase of micro-erosions in gastric mucous; production of lactoferrins by neutrophils; and capture of iron by the bacteria.¹⁵ Another way could be the rise in the synthesis of hepcidin, an essential regulator of iron metabolism that inhibits iron absorption in the small intestine.¹⁶

Therefore, we aimed to determine the prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients.

Materials and Methods

Study Area

The study was carried out at Kiryandongo General Hospital which is located in Kiryandongo District. The hospital is approximately 225 km from Kampala along the Kampala-Gulu highway. It is the biggest public hospital along the Kampala-Gulu highway serving Kiryandongo and parts of Masindi, Nakasongola, Apac, Amuru, and Oyam districts with an estimated catchment population of 47,155 people. Kiryandongo General Hospital laboratory is accredited by the South African National Accreditation System (SANAS) with the capacity to diagnose *H. pylori* infection and anemia.

Study Design and Period

This was a prospective cross-sectional study conducted from November 2021 to February 2022.

Sample Size Calculation

This was done according to a similar study carried out in Southwest Ethiopia where the prevalence of anemia was 24.3%¹⁷ using the Kish and Leslie formula.

$$n = Z^2 P(1-P)/d^2 \quad (1)$$

Where.

n = the desired sample size.

Z = critical values of normal distribution at 95%, which corresponds to 1.96

P = the proportion of the target population estimated to have *H. pylori* infection and anemia 24.3%

Hence n = 283 study participants.

Sampling Technique

A random sampling procedure was done where every adult dyspeptic patient identified was considered for the study. Study participants who met the inclusion criteria were allowed to pick numbers from the box and those that picked odd numbers were enrolled in the study.

Selection Criteria

All adult dyspeptic patients aged 18 years and above attending Kiryandongo General Hospital who consented to participate in the study were included. Patients who had undergone gastrectomy and iron supplements were excluded from the study. Adults who had a history of chronic disease or severely ill patients were also excluded from the study.

Data Collection

Demographic data (age, sex, occupation, household income, marital status, level of education) and risk factors associated with anemia, dietary supplements, history of alcohol consumption, deworming status and drinking boiled or treated water were collected from the participants using a questionnaire.

Dyspepsia was defined as upper abdominal discomfort, often chronic or persistent indigestion, and symptoms include fullness, bloating, nausea, loss of appetite or upper abdominal pain. All study participants with the above symptoms were enrolled in the study.

Four mL (4mL) of the venous blood sample was then collected from each dyspeptic patient into ethylenediamine tetraacetic acid (EDTA) vacutainers. Complete blood count (CBC) was determined using an automated hematology analyzer (HUMA COUNT 30^{TS}, Germany). Thin blood films were made on all blood samples with anemia (Hb concentration <12g/dl for females and <13g/dl for males). Thin and thick blood films were made, thin films were fixed with absolute methanol, and both were stained using Giemsa stain for 20 minutes. Thick films were examined at $\times 1000$ magnification for malaria parasites, and thin films were examined for morphological classification of anemia. Sterile stool containers were given to the participants with an explanation on how to collect approximately 1 gram of stool specimen and tested for the presence of *H. pylori* by stool antigen rapid test strips (Whole Power *H. pylori* antigen rapid test device, Zhejiang Orient Gene Biotech Co, LTD, China) with the sensitivity and specificity of >95 and 95.7%, respectively. Saline stool preparations were also done and examined for the presence of intestinal parasites.

Data Analysis

Data collected were entered into an Excel spreadsheet, cleaned and checked for completeness and then exported to STATA software version 14 for analysis. Demographic data were analyzed and presented in the form of percentages and frequencies. The association between *H. pylori* infection and anemia was studied using Pearson's chi-squared test. Association between *H. pylori* infection and risk factors was studied using logistic regression. Odds ratio >1 and p-value ≤ 0.05 were considered statistically significant.

Ethical Consideration

This study was approved by the Faculty Research Committee (FRC) of Mbarara University of Science and Technology with approval number (MUST/MLS/30). Ethical clearance was also got from the management of Kiryandongo General Hospital before conducting the study. Informed consent was obtained from all study participants before their involvement in the study and was confirmed by their signature or thumbprint on the consent form. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (1964). Confidentiality of the study participants was observed by giving each participant a study code that was not traceable to them. Participants were also informed that their participation is free and voluntary and that they had a right to withdraw from the study at any time and that their withdrawal would not affect their access to medical care.

Results

Socio-Demographic Characteristics of Study Participants

We recruited 283 dyspeptic patients, and 193 (68.2%) were females. The mean age of the study participants was 31.9 years and a standard deviation of 12.7 years. The majority of them were aged 18–22 years (26.8%), had primary education (53.7%), were living in the rural area (73.5%), were not smoking (97.2%), were not taking alcohol (88%) and were drinking piped water (52.3%) as indicated in Table 1.

Prevalence of *H. pylori* Infection Among Dyspeptic Patients

The overall prevalence of *H. pylori* infection was 42.4% (120/283) (Figure 1).

Table I Baseline Characteristics of the Study Participants

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	90	31.8
	Female	193	68.2
Age (years)	18–22	76	26.8
	23–27	51	18.0
	28–32	52	18.4
	33–37	32	11.3
	38–42	22	7.8
	≥42	50	17.7
Education level	None	30	10.6
	Primary	152	53.7
	Secondary	87	30.7
	Tertiary	14	5.0
Residence	Rural	208	73.5
	Urban	75	26.5
Smoking	No	275	97.2
	Yes	8	2.8
Alcohol intake	No	249	88.0
	Yes	34	12.0
Water source	Piped	148	52.3
	Spring	107	37.8
	Open	28	9.9
	water		

Prevalence of Anemia Among Dyspeptic Patients and Its Association with *H. pylori* Infection

The prevalence of anemia among dyspeptic patients was 19.8% (56/283). The mean hemoglobin was 13.9 g/dL with a Standard Deviation (SD) of 2.7 g/dL. Mean hemoglobin among males and females was 14.9 g/dL and 13.5 g/dL with SD of 2.7 g/dL and 2.6 g/dL, respectively. However, the prevalence of anemia among *H. pylori*-infected individuals was higher at 25.8% (31/120) compared to *H. pylori*-negative patient counterparts at 15.3% (25/163). The difference in the prevalence of anemia among *H. pylori* patients and uninfected patients was statistically significant with a Pearson chi-squared value of 4.8 and a p-value of 0.029 (Table 2).

Morphological Classification of Anemia Among *H. pylori*-Infected Dyspeptic Patients

The majority of the *H. pylori*-infected patients who were anemic had microcytic hypochromic anemia 19 (61.3%), followed by normocytic normochromic anemia 5 (16.1%), macrocytic normochromic anemia 4 (12.9%) and macrocytic hypochromic anemia 3 (9.7%) as shown in Figure 2.

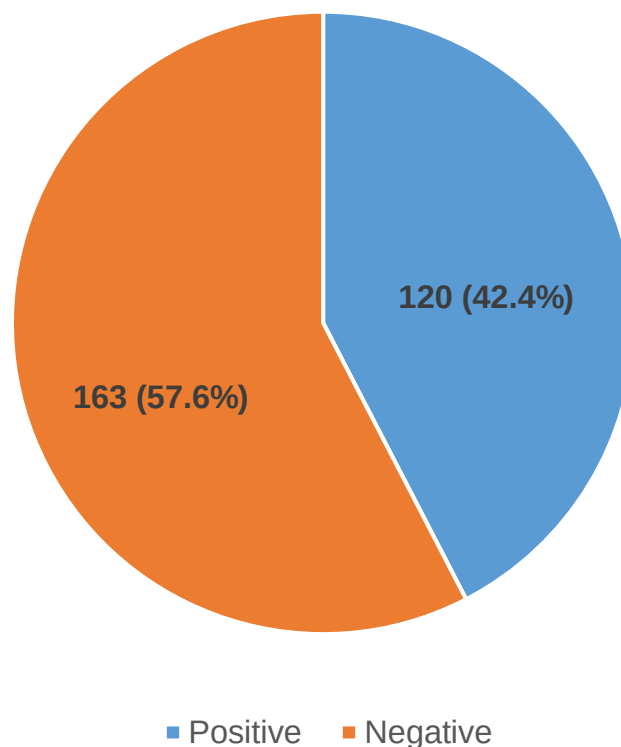


Figure 1 Pie chart showing prevalence of *H. pylori* infection among adult dyspeptic patients at Kiryandongo General Hospital.

Factors Associated with *H. pylori* Infection Among Dyspeptic Patients

Bivariate Analysis of Factors Associated with *H. pylori* Infection

On bivariate analysis, age ($p = 0.08$ and 0.009), and stool Parasitemia ($p = 0.027$) were associated with *H. pylori* infection (Table 3).

Multivariate Analysis of Factors Associated with *H. pylori* Infection

On multivariate analysis, age ($p = 0.020$ and 0.009), water source ($p = 0.049$) and stool Parasitemia ($p = 0.020$) were found associated with *H. pylori* infection as shown in Table 4.

Discussion

Prevalence of Anemia and *H. pylori* Infection Among Dyspeptic Patients

In our study, the overall prevalence of anemia was 19.8% and *H. pylori* infection among dyspeptic patients was 42.4%. However, the prevalence of anemia among *H. pylori*-infected individuals was higher at 11% compared to *H. pylori*-negative patient counterparts at 8.8%. This prevalence is higher than the reported prevalence of *H. pylori* infection in Bwera Hospital in Kasese, Uganda, by Tsongo et al at 29.9%,¹⁸ in Kampala 35.7%,¹⁹ 27.3% in eastern Uganda²⁰ and 29.2% in Butembe Health Centre III Kyankwanzi district, Uganda.

Table 2 A 2×2 Dyspeptic Patients with *H. pylori* Infection in Association with Anemia

	<i>H. pylori</i> -Negative	<i>H. pylori</i> -Positive	Total	χ^2 (p-value)
Non-anemic	138	89	227	4.8 (0.029)
Anemic	25	31	56	
Total	163	120	283	

Note: Chi-square 4.8 and p-value 0.029.

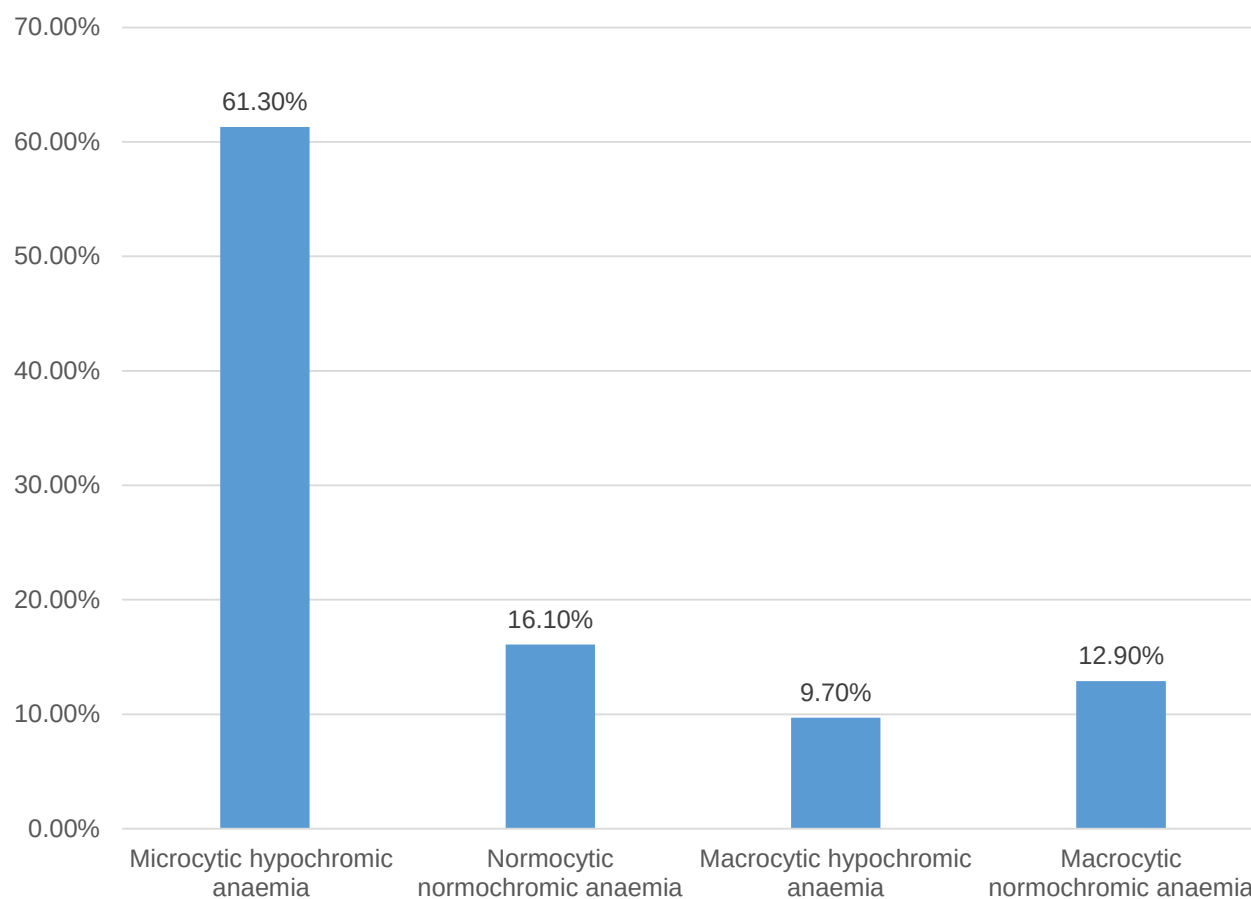


Figure 2 *H. pylori* infected patients with different morphological types of anemia.

Our study finding is not comparable with a study finding from China that found that the burden of *H. pylori* infection was 35.3%²¹ but consistent with a study by Baingana et al in Uganda 45.2%⁷ in Nairobi 46.2%²² 43.9% reported in China²³ and 41% observed in the United Arab Emirates.⁶ Our study finding is lower than the reported prevalence of *H. pylori* infection among dyspeptic patients from Ethiopia 52.4%,²⁴ Nigeria 67.4%,²⁵ and Ibadan 63.5%.²⁶ The observed variation in the burden of *H. pylori* infection is probably attributed to the lack of clear cut-off for dyspepsia, different diagnostic methods used, sample size, and socioeconomic factors.

Association of Anemia and *H. pylori* Infection

Anemia was significantly associated with *H. pylori* with a Chi-squared value of 4.8 and a p-value of 0.029. It also showed at a multivariate level that those who were infected with *H. pylori* were 1.92 times more likely to be anemic than the *H. pylori*-negative group. This finding is in line with findings from Ethiopia,²⁴ and China²³ that observed an association of *H. pylori* infection with anemia. Another study from China showed that *H. pylori* patients had a 2.53 times increased risk of being anemic compared to the *H. pylori*-negative group.²¹ Our finding is however not in line with other scholars' findings from Latin America²⁷ and the rural Haitian population²⁸ that reported no association between *H. pylori* and anemia. This could be due to the sample size studied, geographical variation of the disease, and methods used in the diagnosis of *H. pylori* and anemia.

This study observed that *H. pylori* infection was significantly associated with middle age (33–42 years old) and intestinal parasites. This finding is consistent with the findings in studies in Ethiopia^{24,29} Kuwait,³⁰ South Africa,³¹ Brazil and in the United Arab Emirates where *H. Pylori* infection was observed to Increase with age³² but it differs with the

Table 3 Bivariate Analysis of Factors Associated with *H. pylori* Infection

Variables		Odds ratio	95% Confidence Interval	P value
Gender	Male	Ref		
	Female	0.87	0.49–1.54	0.634
Age (years)	18–22	Ref		
	23–27	1.44	0.66–3.15	0.366
	28–32	1.96	0.89–4.30	0.094
	33–37	3.48	1.38–8.76	0.008*
	38–42	4.18	1.42–12.26	0.009*
	>42	1.02	0.41–2.53	0.961
Education level	None	Ref		
	Primary	0.40	0.16–1.04	0.060
	Secondary	0.50	0.18–1.41	0.188
	Tertiary	0.34	0.07–1.57	0.167
Boil drinking water	Yes	Ref		
	No	0.75	0.43–1.34	0.334
Washing hands before eating	Yes	Ref		
	No	3.93	0.87–17.72	0.075
Water sources	Piped	Ref		
	Spring	0.61	0.34–1.09	0.097
	Open well	0.50	0.19–1.27	0.144
Washing facility at the toilet	Yes	Ref		
	No	1.33	0.39–4.56	0.641
Residence	Rural	Ref		
	Urban	1.52	0.83–2.80	0.169
Smoking	No	Ref		
	Yes	0.86	0.17–4.25	0.853
Alcohol	No	Ref		
	Yes	0.93	0.40–2.17	0.871
Anemia	Non-Anemic	Ref		
	Anemic	1.53	0.78–3.00	0.212
Stool Parasitemia	No	Ref		
	Yes	11.30	1.31–97.46	0.027*

Note: *Significant factor associated with *H. pylori*.

study by Ahmed et al, in Khartoum³³ that reported *H. pylori* infection and its association in individuals >60 years old and a study in eastern Uganda where age was not significantly associated with *H. pylori* infection.²⁰

A case-control study in Sudan reported a relationship between intestinal parasites and *H. pylori* infection.³⁴ Another study in Venezuela also showed evidence of an association between *H. pylori* infection and intestinal parasitic infections,³⁵ and similar finding had been reported in Ethiopia.³⁶ A study by Zylberberg et al in the US reported that

Table 4 Multivariate Analysis of Factors Associated with *H. pylori* Infection

Variable		Adjusted Odds Ratio	95% Confidence Interval	P value
Age (years)	18–22	Ref		
	23–27	1.37	0.64–2.95	0.416
	28–32	1.91	0.88–4.15	0.100
	33–37	2.85	1.18–6.90	0.020*
	38–42	4.06	1.42–11.63	0.009*
	>42	0.85	0.36–2.05	0.725
Education level	None	Ref		
	Primary	0.42	0.17–1.05	0.064
	Secondary	0.54	0.20–1.48	0.231
	Tertiary	0.42	0.10–1.82	0.247
Water sources	Piped	Ref		
	Spring	0.57	0.33–1.00	0.049*
	Open well	0.55	0.22–1.39	0.207
Washing hands before eating	Yes	Ref		
	No	3.60	0.82–15.72	0.090
Stool Parasitemia	No	Ref		
	Yes	12.34	1.48–103.11	0.020*

Note: *Significant factor associated with *H. pylori*.

H. pylori infected study participants are independently associated with giardiasis,³⁷ and similar finding was reported in Germany.³⁸

This current study observed association between *H. pylori* infection and different water sources. The risk of acquiring *H. pylori* infection seems to be multifactorial and potentially contaminated environmental sources, such as local drinking water, swimming in rivers, or the ingestion of fecally contaminated vegetables have been reported as risk factors for *H. pylori* infection.^{39,40} A study by Khoder et al similarly demonstrated the association between *H. pylori* infection and source of drinking water.⁶ Baingana et al in Uganda demonstrated that *H. pylori* infection was independently associated with using water from public wells, boreholes or springs and from rivers, lakes or streams.⁷ However, the association found in this study does not imply causality but it predicts the risk of the occurrence of the disease among the population with the associated variables.

Morphological Types of Anemia Among *H. pylori* Patients

The major morphological form of anemia in *H. pylori*-infected patients was microcytic hypochromic anemia followed by normocytic normochromic anemia, macrocytic normochromic anemia, and macrocytic hypochromic anemia. This highly suggests that *H. pylori* infection causes iron deficiency by reducing iron absorption from the intestine and causes majorly microcytic hypochromic red blood cells with low hemoglobin concentration due to iron deficiency.²⁴ This is not in line with findings from Ethiopia.¹⁷ They found that the major morphological type of anemia among *H. pylori*-infected patients was normocytic normochromic anemia. Another study found that the major morphological class of anemia among *H. pylori* patients was macrocytic normochromic anemia.²³

Limitations

The cross-sectional design coupled with lack of control population for comparison limited the opportunity to establish the association of *H. pylori* infection and anemia.

Conclusion

The prevalence of *H. pylori* infection reported among dyspeptic patients in the Kiryandongo district in this study was high. *H. pylori* infection was independently associated with anemia, increasing age, water sources and intestinal parasitaemia among dyspeptic patients. Microcytic hypochromic anemia was the commonest morphological type of anemia among dyspeptic patients with *H. pylori* infection. Routine screening of dyspeptic patients for *H. pylori* infection, hemoglobin estimation of the infected patients and improvement of water sources is advised.

Data Sharing Statement

The datasets used in the analysis of this research study are available from the corresponding author upon reasonable request.

Acknowledgments

We acknowledge the study participants, the Clinicians, the Principal Nursing Officer, and the Laboratory staff of Kiryandongo General Hospital for the support they gave us during the study period.

Disclosure

The authors report no conflicts of interest in this work.

References

- Hooi JK, Lai WY, Ng WK., et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. *Gastroenterology*. 2017;153(2):420–429. doi:10.1053/j.gastro.2017.04.022
- Mendoza E, Duque X, Hernández Franco JI, et al. Association between active H. pylori infection and iron deficiency assessed by serum hepcidin levels in school-age children. *Nutrients*. 2019;11(9):2141. doi:10.3390/nu11092141
- Zamani M, Ebrahimitabar F, Zamani V, et al. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. *Aliment Pharmacol Ther*. 2018;47(7):868–876. doi:10.1111/apt.14561
- de Brito BB, da Silva FAF, Soares AS, et al. Pathogenesis and clinical management of Helicobacter pylori gastric infection. *World J Gastroenterol*. 2019;25(37):5578. doi:10.3748/wjg.v25.i37.5578
- Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of Campylobacter infection. *Clin Microbiol Rev*. 2015;28(3):687–720. doi:10.1128/CMR.00006-15
- Khoder G, Muhammad JS, Mahmoud I, Soliman SS, Buruoca C. Prevalence of Helicobacter pylori and its associated factors among healthy asymptomatic residents in the United Arab Emirates. *Pathogens*. 2019;8(2):44. doi:10.3390/pathogens8020044
- Baingana RK, Kiboko Enyaru J, Davidsson L. Helicobacter pylori infection in pregnant women in four districts of Uganda: role of geographic location, education and water sources. *BMC Public Health*. 2014;14(1):1–10. doi:10.1186/1471-2458-14-915
- Campuzano-Maya G. Hematologic manifestations of Helicobacter pylori infection. *World J Gastroenterol*. 2014;20(36):12818. doi:10.3748/wjg.v20.i36.12818
- Malfertheiner P, Selgrad M. Helicobacter pylori infection and current clinical areas of contention. *Curr Opin Gastroenterol*. 2010;26(6):618–623. doi:10.1097/MOG.0b013e32833efede
- Pacifico L, Osborn JF, Tromba V, Romaggioli S, Bascetta S, Chiesa C. Helicobacter pylori infection and extragastric disorders in children: a critical update. *World J Gastroenterol*. 2014;20(6):1379. doi:10.3748/wjg.v20.i6.1379
- Huang X, Qu X, Yan W, et al. Iron deficiency anaemia can be improved after eradication of Helicobacter pylori. *Postgrad Med J*. 2010;86(1015):272–278. doi:10.1136/pgmj.2009.089987
- Wenzhen Y, Yumin L, Kehu Y, et al. Iron deficiency anemia in Helicobacter pylori infection: meta-analysis of randomized controlled trials. *Scand J Gastroenterol*. 2010;45(6):665–676. doi:10.3109/00365521003663670
- Xin-Hua Q. Iron deficiency anemia can be improved after eradication of Helicobacter pylori. *Postgrad Med J*. 2010;86:272–278.
- Hudak L, Jaraisy A, Haj S, Muhsen K. An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anemia. *Helicobacter*. 2017;22(1):e12330. doi:10.1111/hel.12330
- Stein J, Connor S, Virgin G, Ong DEH, Pereyra L. Anemia and iron deficiency in gastrointestinal and liver conditions. *World J Gastroenterol*. 2016;22(35):7908. doi:10.3748/wjg.v22.i35.7908
- Ganz T. Systemic iron homeostasis. *Physiol Rev*. 2013;93(4):1721–1741. doi:10.1152/physrev.00008.2013
- Haile K, Yemane T, Tesfaye G, Wolde D, Timerga A, Haile A. Anemia and its association with Helicobacter pylori infection among adult dyspeptic patients attending Wachemo University Nigist Eleni Mohammad Memorial Referral Hospital, Southwest Ethiopia: a cross-sectional study. *PLoS One*. 2021;16(1):e0245168. doi:10.1371/journal.pone.0245168
- Tsongo L, Nakavuma J, Mugasa C, Kamalha E. Helicobacter pylori among patients with symptoms of gastroduodenal ulcer disease in rural Uganda. *Infect Ecol Epidemiol*. 2015;5(1):26785. doi:10.3402/iee.v5.26785
- Namyalo E, Nyakarahuka L, Afayoa M, et al. Prevalence of Helicobacter pylori among Patients with Gastrointestinal Tract (GIT) Symptoms: a Retrospective Study at Selected Africa Air Rescue (AAR) Clinics in Kampala, Uganda, from 2015 to 2019. *J Trop Med*. 2021;2021:548.
- Nekaka R, Oboth P, Nteziyaremye J, Gavamukulya Y, Ssenyonga LV, Iramiot JS. Sero prevalence and factors associated with Helicobacter pylori infection in a rural population in Eastern Uganda a community cross sectional study. *Primary Health Care*. 2021;11(4):1–9.

21. Hou B, Zhang M, Liu M, et al. Association of active *Helicobacter pylori* infection and anemia in elderly males. *BMC Infect Dis.* 2019;19(1):1–9. doi:10.1186/s12879-019-3849-y
22. Said MK. *Prevalence of Helicobacter Pylori Infection Among Patients with Peptic Ulcers and the Associated Risk Factors in Mbagathi Level V Hospital.* Nairobi County, Kenya: School Of Medicine, Kenyatta University; 2019.
23. Xu M-Y, Cao B, Yuan B-S, Yin J, Liu L, Lu Q-B. Association of anaemia with *Helicobacter pylori* infection: a retrospective study. *Sci Rep.* 2017;7(1):1–7. doi:10.1038/s41598-016-0028-x
24. Kibru D, Gelaw B, Alemu A, Addis Z. *Helicobacter pylori* infection and its association with anemia among adult dyspeptic patients attending Butajira Hospital, Ethiopia. *BMC Infect Dis.* 2014;14(1):1–7. doi:10.1186/s12879-014-0656-3
25. Aje A, Otegbayo J, Odaibo G, Bojuwoye B. Comparative study of stool antigen test and serology for among Nigerian dyspeptic patients-a pilot study. *Niger J Clin Pract.* 2010;13(2):120–124.
26. Jemilohun AC, Otegbayo JA, Ola SO, Oluwasola OA, Akere A. Prevalence of *Helicobacter pylori* among Nigerian patients with dyspepsia in Ibadan. *Pan Af Med J.* 2010;6:1. doi:10.4314/pamj.v6i1.69063
27. Santos IS, Boccio J, Davidsson L, et al. *Helicobacter pylori* is not associated with anaemia in Latin America: results from Argentina, Brazil, Bolivia, Cuba, Mexico and Venezuela. *Public Health Nutr.* 2009;12(10):1862–1870. doi:10.1017/S1368980009004789
28. Shak JR, Sodikoff JB, Speckman RA, et al. Anemia and *Helicobacter pylori* seroreactivity in a rural Haitian population. *Am J Trop Med Hyg.* 2011;85(5):913. doi:10.4269/ajtmh.2011.11-0101
29. Shiferaw G, Abera D. Magnitude of *Helicobacter pylori* and associated risk factors among symptomatic patients attending at Jasmin internal medicine and pediatrics specialized private clinic in Addis Ababa city, Ethiopia. *BMC Infect Dis.* 2019;19(1):1–6. doi:10.1186/s12879-019-3753-5
30. Alazmi WM, Siddique I, Alateeqi N, Al-Nakib B. Prevalence of *Helicobacter pylori* infection among new outpatients with dyspepsia in Kuwait. *BMC Gastroenterol.* 2010;10(1):1–4. doi:10.1186/1471-230X-10-14
31. Tanih N, Okeleye B, Ndip I, et al. *Helicobacter pylori* prevalence in dyspeptic patients in the Eastern Cape Province–race and disease status. *South Af Med J.* 2010;100(11):734–737. doi:10.7196/SAMJ.4041
32. Escobar-Pardo ML, Godoy A, Machado RS, Rodrigues D, Fagundes Neto U, Kawakami E. Prevalence of *Helicobacter pylori* infection and intestinal parasitosis in children of the Xingu Indian Reservation. *J Pediatr.* 2011;87:393–398. doi:10.2223/JPED.2118
33. Ahmed NFM. Prevalence rate of *Giardia lamblia*/*Helicobacter pylori* co-infections in Khartoum state-Sudan. *BMC Public Health.* 2016.
34. Abd Elbagi YY, Abd Alla AB, Saad MBE. The relationship between *Helicobacter pylori* infection and intestinal parasites in individuals from Khartoum state, Sudan: a case-control study. *F1000Research.* 2019;8:8. doi:10.12688/f1000research.17047.1
35. Fuenmayor-Boscán AD, Hernández IM, Valero KJ, Paz AM, Sandra LB, Rivero Z. Association between *Helicobacter pylori* and intestinal parasites in an Añu indigenous community of Venezuela. *Indian J Gastroenterol.* 2016;35(2):106–112. doi:10.1007/s12664-016-0641-4
36. Seid A, Tamir Z, Ksanew B, Senbetay M. Co-infection of intestinal parasites and *Helicobacter pylori* among upper gastrointestinal symptomatic adult patients attending Mekanaselem Hospital, northeast Ethiopia. *BMC Res Notes.* 2018;11(1):1–6. doi:10.1186/s13104-018-3246-4
37. Zylberberg HM, Green PH, Turner KO, Genta RM, Lebwohl B. Prevalence and predictors of giardia in the United States. *Dig Dis Sci.* 2017;62(2):432–440. doi:10.1007/s10620-016-4447-0
38. Espelage W, Stark K, Alpers K. Characteristics and risk factors for symptomatic *Giardia lamblia* infections in Germany. *BMC Public Health.* 2010;10(1):1–9. doi:10.1186/1471-2458-10-41
39. Leja MAA, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter.* 2016;21(3–7):2016. doi:10.1111/hel.12332
40. Samra ZQ, Javaid U, Ghafoor S, Batool A, Dar N, Athar MA. PCR assay targeting virulence genes of *Helicobacter pylori* isolated from drinking water and clinical samples in Lahore metropolitan, Pakistan. *J Water Health.* 2011;9(1):208–216. doi:10.2166/wh.2010.169

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Serum Ferritin Levels and Other Associated Parameters with Diabetes Mellitus in Adult Patients Suffering from Beta Thalassemia Major

Ahmed Saleh Ibrahim, Azza Hasan Abd El-Fatah, Ayman Fathy Abd El-Halim & Farid Fawzy Mohamed

To cite this article: Ahmed Saleh Ibrahim, Azza Hasan Abd El-Fatah, Ayman Fathy Abd El-Halim & Farid Fawzy Mohamed (2023) Serum Ferritin Levels and Other Associated Parameters with Diabetes Mellitus in Adult Patients Suffering from Beta Thalassemia Major, Journal of Blood Medicine, , 67-81, DOI: [10.2147/JBM.S390666](https://doi.org/10.2147/JBM.S390666)

To link to this article: <https://doi.org/10.2147/JBM.S390666>



© 2023 Ibrahim et al.



Published online: 07 Feb 2023.



Submit your article to this journal



Article views: 509



View related articles



View Crossmark data



Citing articles: 5 View citing articles

Serum Ferritin Levels and Other Associated Parameters with Diabetes Mellitus in Adult Patients Suffering from Beta Thalassemia Major

Ahmed Saleh Ibrahim¹, Azza Hasan Abd El-Fatah², Ayman Fathy Abd El-Halim³, Farid Fawzy Mohamed²

¹Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt; ²Department of Internal Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt; ³Department of Hematology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Correspondence: Ahmed Saleh Ibrahim, Tel +20 109 232 6689, Email abadawi70@yahoo.com

Background: Although beta thalassemia major (BTM) patients are properly treated with blood transfusions in accompany with iron chelation therapy, they suffer from complications, such as diabetes mellitus (DM).

Purpose: The purpose was to detect the critical serum ferritin level and other parameters correlated with DM among adult BTM patients. Also, it was to study whether each of these parameters is associated with a certain period of age.

Patients and Methods: This study included 200 adult BTM patients. A cross-sectional study was carried out. Patients clinical and laboratory variables, such as ferritin levels, and fasting blood glucose (FBS) were extracted from medical records at Zagazig University Hospital, Egypt. Liver and cardiac iron contents were assessed using MRI T2* methods. Statistical analysis was performed using IBM SPSS V26.0 software package.

Results: The overall frequency of DM over the total sample equals 6.5%. There were no impaired fasting glucose (IFG) in the medical records. Statistical significance between serum ferritin and DM was ($P = 0.014$). The serum ferritin 2500 ng/mL with age group (27–<32) years-old were risk factors. The distributions of DM according to BMI were (3.5%) of class overweight. Significant association between DM and BMI was ($r = 0.357$, $P < 0.001$). Liver MRI T2* has significant correlation with serum ferritin, but cardiac MRI T2* was poorly correlated. Association between liver and cardiac MRI T2* was not found.

Conclusion: Age group (27–<32) years-old and ferritin >2500 ng/mL should be properly treated immediately. The serum ferritin and BMI of class “overweight” were risk factors for DM. Factors such as diet should be followed. Serum ferritin can be used for estimating liver iron content for economic factors. But cardiac MRI T2* must be performed for evaluating cardiac iron accurately.

Keywords: hemoglobin, iron chelation, blood glucose, endocrinopathy

Introduction

Frequency of thalassemia patients with diabetes mellitus lies in the range 20% to 30% worldwide as stated in the literature. Some researchers in a multicenter reported that the prevalence of diabetes mellitus (DM) among thalassemia major patients was 4.9% (sample size 1861). In another work, diabetes was detected in 17% of beta thalassemia major patients.^{1,2}

Although the management of beta thalassemia major patients with frequent blood transfusion and iron chelation treatments, there was an excess of iron deposition in beta cells of pancreas. This leads to destruction of pancreatic beta cells and deficiency of insulin secretion^{3,4} so, treating with insulin is necessary. Iron chelation treatment with desferrioxamine (DFO) and deferiprone were used to reverse glucose intolerance and to delay the beginning of diabetes.⁵

Recent works reported that deferasirox was effective for BTM patients. Moreover, physical activity and weight reduction are beneficial for preventing the development of diabetes. The degree of destruction of beta cells and hence reduction of insulin secretion are key factors determining the management of diabetes.^{6–8} two systematic plan for

treatment. First, premixed insulin is used before breakfast and dinner. Second, basal slow acting insulin once daily (eg, levemir or glargine) and prandial rapid acting insulin prescribed with each meal.⁹

Motivations for this research can be summarized as follows: First to get more information on the prevalence of the studied endocrine complication because there is a lack of information in our area. Second, to be able to install a database system for those BTM patients as a foundation stone of a whole database network in Egypt. Finally, to clarify the underlying endocrine disorder (eg, DM in this work) for optimal management and treatments.

Materials and Methods

The current work is cross-sectional study. The study depends on collecting data reported in the patient's medical reports including hematologists and endocrinologists as well as biochemical laboratory checkup. The assessment was done on the data of the patients with beta-thalassemia major attending outpatient clinic of Zagazig University Hospitals.

The patients with beta thalassemia major were enrolled and their medical records were studied following the inclusion and exclusion criteria. The number of records for patients fulfilling the inclusion criteria from 2016 to 2020 was 200 records. Patients ≥ 18 years old were considered in the study. Patients < 18 years old, and patients with hemolytic anemia other than beta-thalassemia major were excluded. Also, patients who died were deleted from patient's records; subsequently, 40 patients were excluded and 200 patients remained in the study. So, two hundred patients from Zagazig University Hospitals, with confirmed diagnosis of beta-thalassemia major entered in the study. The patients were classified according to age into the following groups: First group: 18–<22 years old; Second group: 22–<27 years old; Third group: 27–< 32 years old; Forth group: ≥ 32 years old.

Inclusion and exclusion criteria: patients diagnosed with beta-thalassemia major with ages ≥ 18 years old were included in the study. Also, both sex are considered. The exclusion criteria was specified as follows: Patients with chronic hemolytic anemia other than beta-thalassemia major were excluded. Moreover, patients with ages < 18 years old were not considered in the study.

Data collection: all patients' records must include the following items: (a) Full medical history and through physical examination reported in the medical files. (b) Assessment of anthropometric measures, such as length, weight, body mass index, etc. (c) Routine investigations according to our local standards, eg, serum ferritin, liver function tests, kidney function tests, etc.

Biochemical laboratory data: serum calcium (Ca), phosphorus (P), serum 25-OH vitamin D and fasting blood sugar (FBS) were assessed and serum ferritin levels were measured and collected.

Body measurements and body mass index: Body mass index (BMI) was invented by Prof. Adolphe Quetelet in 1830. Height was measured by a standard wall-mounted meter and rounded to the nearest 0.5 cm. The weight was assessed via a standard scale, while the patient was wearing light clothes with no shoes. Using the standard formula, BMI was calculated as follows:

$$\text{BMI} = \text{weight}(\text{in kg}) / \text{height}(\text{in m}^2)$$

BMI was classified into the following classes: underweight (BMI < 18.5), healthy (18.5–<25), overweight (25–<30), and obese (BMI ≥ 30).¹¹

Diagnostic Criteria

Diabetes mellitus (DM) diagnosis was based on the American Diabetes Association criteria, which defines impaired fasting glucose (IFG) for FBS 100–125 mg/dl, also known as pre-diabetic and DM in patients with FBS ≥ 126 mg/dl (American diabetes association ADA2018).¹⁰

Magnetic Resonance Imaging (MRI T2*)

MRI T2* was performed using Magneto, 1.5 T, Siemens medical systems. The measurements of liver and cardiac MRI T2* were included. The cut-off time values that showed the degree of severity of iron overload in the heart and liver were as follows:³⁷

For liver: Normal ≥ 6.3 milliseconds (msec.), mild 2.8 - <6.3 msec, moderate 1.4 - <2.8 msec, and severe <1.4 msec.

For heart: Normal ≥ 20 milliseconds (msec.), mild 14 - < 20 msec. moderate 10 - < 14 msec. and severe < 10 msec. The correlation between serum ferritin levels with both the liver and cardiac iron by MRI T2* were assessed.

Statistical Analysis

Data were analyzed by SPSS software package version 26. Descriptive data are presented as mean, standard deviation, and percentages. Inferential analysis was done using Chi square test to compare categorical variables among different groups, Student's *t*-test to compare quantitative variables between the two groups, and ANOVA test to compare quantitative variables among three or more groups.

The associated variables with DM were investigated to determine their correlations with that endocrinopathy. In this work, we suggested the correlated factors including age, ferritin level, BMI, gender and hemoglobin (Hb). Correlation and bivariate analysis of DM with those factors were studied. A two-tailed P-value < 0.05 was considered to be statistically significant.

Results

A total number of 200 β -thalassemia major patients were enrolled in this study. The general sociodemographic and medical characteristics of the sample are shown in Table 1. The mean serum ferritin level among BTM patients was 3909.0200 ± 1935.72465 (ng/mL). Also, the mean of body mass index BMI (kg/m^2) was 20.4995 ± 3.12525 .

The study sample with beta-thalassemia major (BTM) patients with the total mean age over the whole sample N (200 patients) was 24.6840 ± 5.30761 (Table 2). The patients were 115 males and the 85 females. The gender distribution over the sample N is given in (Table 3) where 57.5% represents males and 42.5% females.

Table 1 General Characteristics and Laboratory Data of the Patients in This Study

Variables	N	Mean	Median	Standard Deviation
Age (years)	200	24.6840	23.5000	5.30761
Weight (kg)	200	53.9950	56.0000	11.52733
Length (cm)	200	161.6500	162.5000	11.33046
Gender: F/M (115/ 85)	200	—	—	—
BMI (kg/m^2)	200	20.4995	20.3800	3.12525
Hb (g/dL)	200	7.1290	7.1000	0.91386
S.Ferritin (ng/mL)	200	3909.0200	3410.0000	1935.72465
S.calcium (mg/dL)	200	7.9371	8.1000	1.30125
S. phosph (mg/dL)	200	4.9985	4.9450	0.99161
Vitamin D (ng/mL)	200	19.6673	18.3000	9.57021
ALT (U/L)	200	31.1830	27.0000	23.69074
AST (U/L)	200	32.1016	30.6000	13.42624
Cr (mg/dL)	200	0.7742	0.6650	0.36617
Iron Chelation Therapy (ICT)	n/N			
Deferoxamine	24/200			
Deferasirox	33/200			
Combined	17/200			
Splenectomy	20/200			

Table 2 Mean Age Distribution

Mean Age				
		Mean	N	Std. Deviation
Valid	Male	24.9678	115	5.53956
	Female	24.3000	85	4.98316
	Total	24.6840	200	5.30761

Table 3 Gender Distribution

Gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	115	57.5	57.5	57.5
	Female	85	42.5	42.5	100.0
	Total	200	100.0	100.0	

The distribution of age groups are also given in [Table 4](#). In the age group (18 - <22) years old the frequency was 85 (42.5%). The occurrence of patients was 55 (27.5%) in the age group (22 - <27) years old. Also, the frequency of patients was 43 (21.5%) in the age group (27-<32) years old. For the age group (≥ 32) years old, the frequency was 17 (8.5%).

The serum ferritin levels among BTM patients are divided into four ranges ([Table 5](#)) as follows: (0–1500 ng/mL); ferritin level range (1501–2500 ng/mL); ferritin level (2501–3500 ng/mL); and ferritin level range (>3500 ng/mL). It

Table 4 Age Groups Distribution

Age Groups (Years)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18 -<22	85	42.5	42.5	42.5
	22-<27	55	27.5	27.5	70.0
	27-<32	43	21.5	21.5	91.5
	≥ 32	17	8.5	8.5	100.0
	Total	200	100.0	100.0	

Table 5 Distribution of Ferritin Levels Among BTM Patients

Ferritin Level (ng/mL)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0–1500	20	10.0	10.0	10.0
	1501–2500	35	17.5	17.5	27.5
	2501–3500	49	24.5	24.5	52.0
	>3500	96	48.0	48.0	100.0
	Total	200	100.0	100.0	

should be noted that iron chelation therapy (ICT) and splenectomy were not completely recorded in the patients' medical records.

Distribution of Serum Ferritin Levels Among BTM Patients

The distribution of ferritin level was given in (Table 5). The frequency of patients was 20 (10%) had serum ferritin level range (0–1500) ng/mL. Also, 35 (17.5%) patients were in the ferritin level range (1501–2500) ng/mL. In the range (2501–3500) ng/mL the patients were 49 (24.5) %. The frequency of patients was 96 (48%) found in the ferritin level range (>3500) ng/mL. This information reflected poor compliance with iron chelation therapy (ICT), and the patients did not follow up the instruction regarding monitoring the ferritin level and continuously taking ICT. Also, this might be happened because of patients' economic reasons.

Iron chelation therapy (ICT) for BTM patients was not completely recorded in the medical records. It was found that 24 patients took deferoxamine, while 33 patients prescribed deferasirox and 17 followed combined ICT. Moreover, splenectomy was not completely registered in the patient files.

The body mass index (BMI) distribution among the patients with beta –thalassemia major in the studied sample was as follows: one hundred thirty four of the patients 134 (67%) had normal BMI while the others had underweight 47 (23.5%) and overweight were 19 (9.5%) in (Table 6 and Figure 1). There were no obese patients in the sample under consideration (N =200).

General characteristics, laboratory data, and more information about distribution of age, gender, ferritin levels and body mass index are summarized in the (Table 1).

Distribution of Diabetes Mellitus Among BTM Patients According to Certain Parameters and Its Correlation

The overall frequency of diabetes mellitus among the BTM patients over the total sample size (N=200) was 6.5%. This result was represented visually in (Figure 2). Distribution of DM according to the age groups was studied. The maximum frequency was 2.5% found in the age group (27–<32) years old, while the minimum frequency 1% was found in the age group (18–<22) years old. It was noted that a frequency of (1.5%) was found in each of the age group (22–<27) years old and the age group (≥32) years.

According to the ferritin level, the maximum frequency of this endocrine dysfunction was 9 (4.5%) that found in patients with ferritin level range >3500 ng/mL (Figure 3). The median frequency was 1.5% with the associated ferritin level range (2501–3500) ng/mL. It was shown from (Figure 3) that the minimum serum ferritin level range at which DM had appeared was (1501–2500 ng/mL). So, the upper bound of this range was 2500 ng/mL which can be considered as a risk factor.

The body mass index (BMI) was studied with this endocrine disorder. It was found that the maximum frequency of that endocrinopathy was 7 (3.5%) found in the class of overweight patients (Figure 4). Those patients (their number was 7) need physical activity and weight reduction besides the treatment of DM. The minimum frequency of DM was 3% with BMI of class normal weight.

Study of DM in lean groups (underweight and normal weight classes) among adult BTM patients was given (Table 7). Underweight group with DM was not found in the studied samples, only diabetic normal weight was detected. In the lean

Table 6 Body Mass Index Distribution

		Frequency	Percent
Valid	Under weight	47	23.5
	Normal weight	134	67.0
	Over weight	19	9.5
	Total	200	100.0

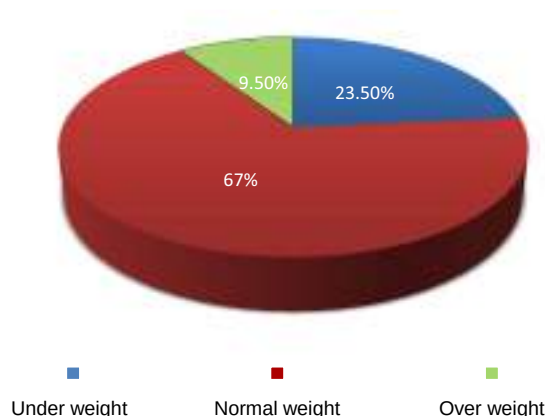


Figure 1 Frequency distribution of body mass index.

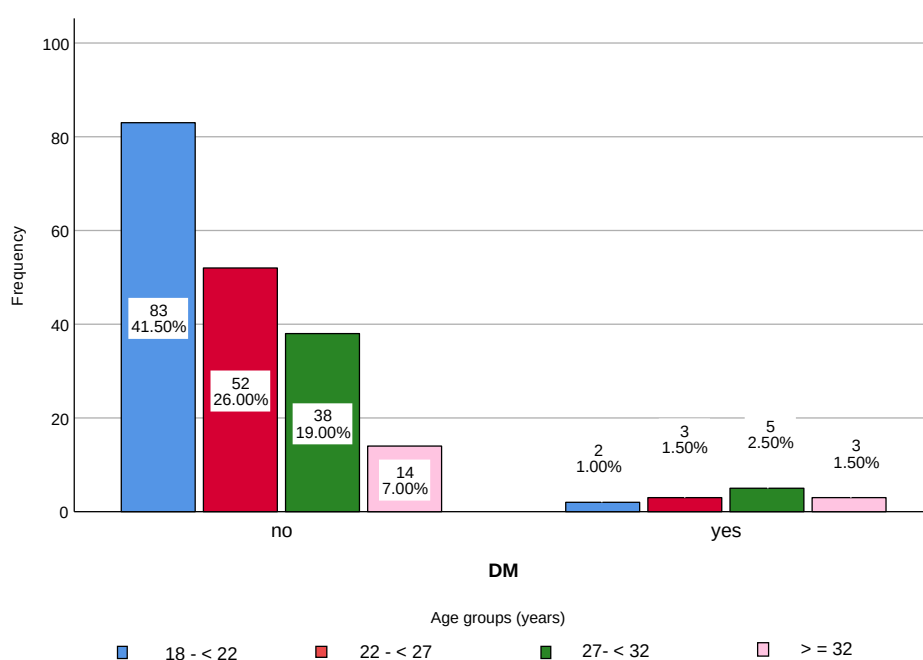


Figure 2 Distribution of Diabetes mellitus among the patients according to age groups.

groups with and without DM, the mean of ages (years), BMI (kg/m^2), ferritin levels (ng/mL) and fasting blood glucose (mg/dL) were computed.

Using bivariate statistical method, it was detected a high correlation between DM and serum ferritin in the group with BMI of class normal weight ($r = 0.912$, $P = 0.011$). But in the normal weight group and underweight one without DM, weak correlations with ferritin ($r = 0.295$) and ($r = 0.178$) was found, respectively. This interpreted that serum ferritin was the risk factor in the lean diabetic group with minimum ferritin level was $2600 \text{ ng}/\text{mL}$ (Table 7).

Moreover, the distribution of DM according to gender was given in (Figure 5). The maximum frequency of DM was 4% found in females, while the frequency of 2.5% found in males.

DM and the Correlated Parameters

Using the correlation analysis with the bivariate method and Pearson correlation, BMI was statistical significance with that endocrine dysfunction ($P < 0.001$), and given in (Table 8). Also, it was found that there was an association between

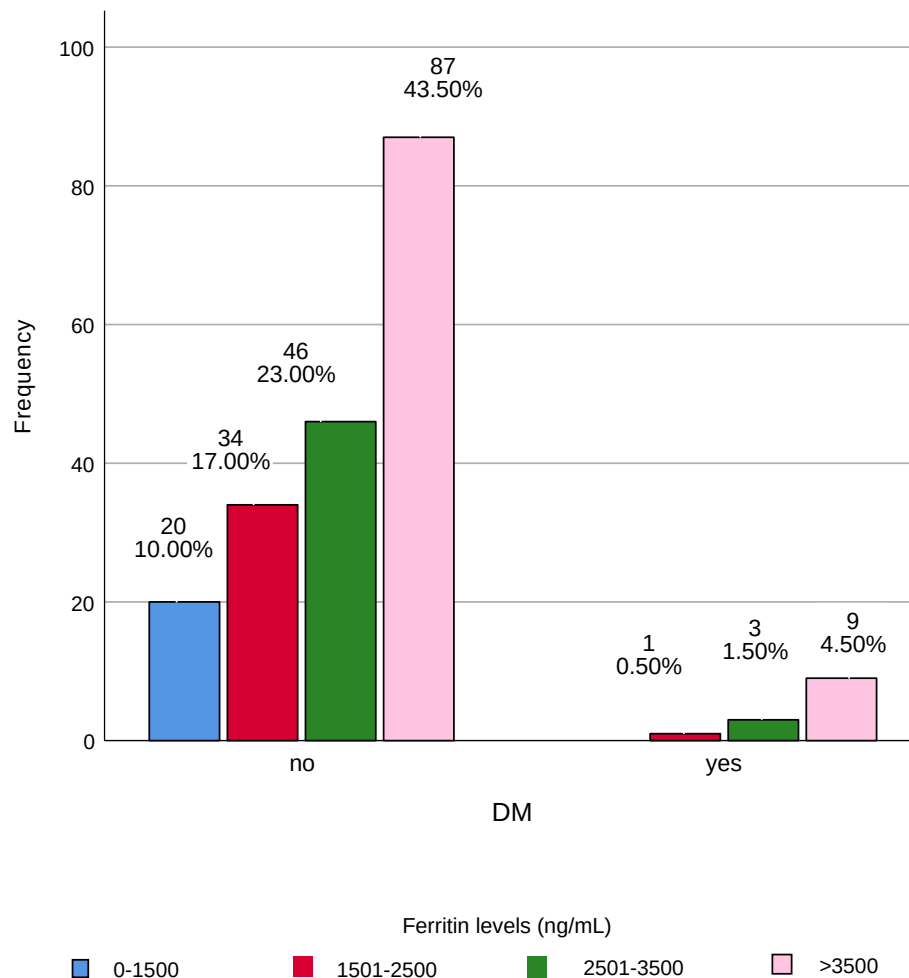


Figure 3 Distribution of Diabetes mellitus among the patients with different ferritin levels.

serum ferritin level and DM ($P = 0.014$). From the previous discussion and the results given in (Figure 3), it can be concluded that the serum ferritin level 2500 ng/mL can be considered as a risk factor of that endocrinopathy.

Also, from Table 8, the statistical P -value of the patients' age groups was ($P = 0.006$). Also, the most prevalent of DM was (2.5%) in the age group (27–<32) years old. So, this parameter was statistically significant. However, there was no association between DM and gender. Also, it was found that DM had no correlation with Hb.

Correlation Between Serum Ferritin and Both Liver and Cardiac Magnetic Resonance Imaging –T2-Star (MRI T2*)

The correlations between serum ferritin levels and both liver and cardiac MRI T2* are shown in Table 9. There was a moderate negative correlation between liver MRI T2* and serum ferritin readings ($r = -0.681$, $P < 0.001$) (Table 9 and Figure 6). Also, there was a negative poor correlation between cardiac MRI T2* and serum ferritin level ($r = -0.284$, $P = 0.002$) (Table 9 and Figure 7). The poor correlation between heart MRI T2* and serum ferritin readings reflected that the serum ferritin level could not predict the heart iron contents. This result ensures the importance of MRI T2* as a more accurate technique for predicting the cardiac iron overload. The moderate correlation between liver MRI T2* and serum ferritin level, which had statistical significance ($P < 0.001$) proved that the serum ferritin can be applied for estimating the liver iron level for economic factors.

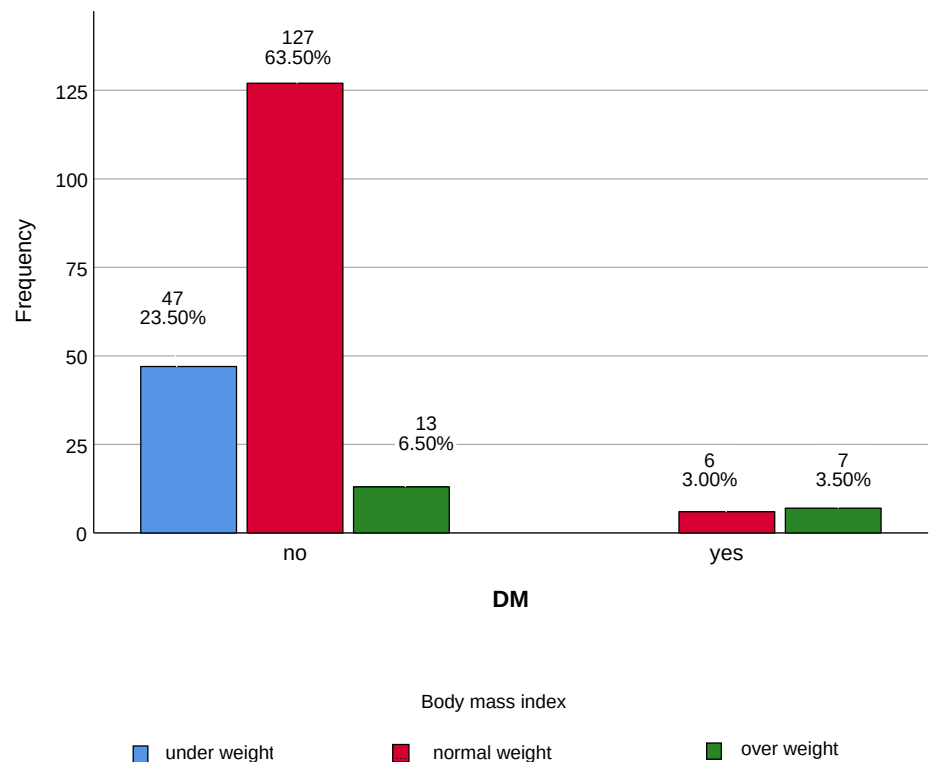


Figure 4 Distribution of Diabetes mellitus among the patients with different classes of BMI.

From Table 10, the correlation between cardiac MRI T2* and liver MRI T2* was ($r=0.064$, $p=0.367$). This means that there was a very poor correlation between cardiac and liver MRI T2* readings among BTM patients with no statistical significance. These results were similar to other studies.^{38,39} This important result emphasizes the role of cardiac MRI T2* for early predicting the heart iron contents among thalassemia patients instead of depending only on liver MRI T2*.

Discussion

In this work, we evaluated the prevalence and associated parameters which can be considered as risk factors with DM. It showed that the overall frequency of diabetes mellitus (DM) in adult beta thalassemia major patients greater than or equal

Table 7 Study of DM Among Lean Groups of Adult BTM Patients

Variables		Lean Group With DM (DM=Yes) (Underweight Group with DM not Found)	Lean Groups Without DM (DM=No)	
		Normal Weight	Underweight	Normal Weight
Age	Mean ±SD	29.5500±3.9098	21.1323±2.3794	25.0654±4.3367
(years)	Min.	25.00	18.30	18.00
	Max.	35.60	26.50	32.00
BMI (kg/m ²)	Mean ±SD	22.0083±1.5822	16.7560±1.2898	20.7257±1.3470
	Min.	20.30	14.20	18.60
	Max.	24.70	18.30	23.50

(Continued)

Table 7 (Continued).

Variables		Lean Group With DM (DM=Yes) (Underweight Group with DM not Found)	Lean Groups Without DM (DM=No)	
		Normal Weight	Underweight	Normal Weight
S.Ferritin (ng/mL)	Mean $\pm$ SD	5926.8333 $\pm$ 2323.5822*	2190.8723 $\pm$ 425.143	2691.2913 $\pm$ 709.010
	Min.	2600.00	1268.00	1120.00
	Max.	9863.00	2900.00	3980.00
Fasting Blood glucose (mg/dL)	Mean $\pm$ SD	168.6667 $\pm$ 20.2649*	115.2553 $\pm$ 4.05113	116.7244 $\pm$ 5.8141
	Min.	137.00	104.00	103.00
	Max.	195.00	121.00	124.00

Note: *Statistically significant at P -value < 0.05.

18 years old was 6.5% with the mean age (24.6840 $\pm$ 5.30761). In other studies, DM has been reported with frequency of 3–26% in BTM patients.^{6,19,20} DM has also been reported with frequencies of 10.5% in United Arab Emirates,²¹ 9.4% in Brazil,²² 15.9% in Iran.¹²

In Italian working group on endocrine complications (sample size was 1861),^{23,24} the overall frequency of diabetes mellitus was 4.9%. Furthermore, and the frequency of DM in the age group (18–30 years old) was much more prevalent than in the age group >30 years (approximately double).²³ This result was in direction with our result where the frequency of age group (18–32 years) was 5% (Figure 2) and in the age group (>32 years) 1.5%, ie, the frequency was approximately 3.3 times of the latter. This may be due to a number of patients with thalassemia major had been died after age >32 years. So, the frequency of DM in last age group was decreased. It should be noted that, the wide range of variation in prevalence depends on the patients' age, duration of red blood cells transfusion, iron overload, organ disorder, iron chelation protocol, compliance to treatment.^{6,25}

In 2017, a published work reported that the prevalence of diabetes mellitus among adult beta- thalassemia major patients is greater than or equal to 18 years (4/56) 7.1%, which is close to our result.²⁶ In 2010, a work

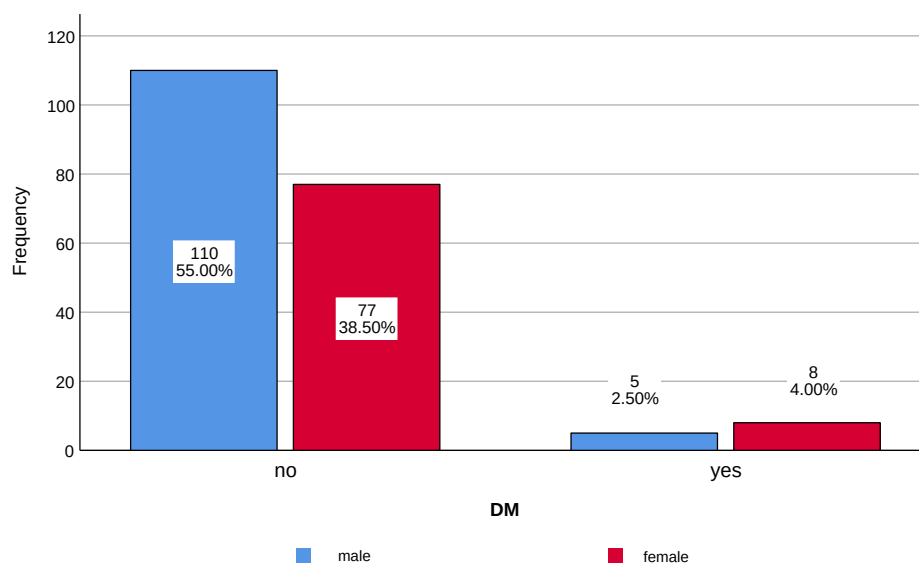


Figure 5 Distribution of Diabetes mellitus among the patients according to gender.

Table 8 Association Between DM and Various Parameters

		Age groups	BMI	Gender	Hb	S. Ferritin
DM	Pearson Correlation	0.195**	0.357**	0.102	0.004	0.174*
	Significance (2-tailed)	0.006	< 0.001	0.153	0.951	0.014
	N	200	200	200	200	200

Notes: *Statistically significant at P-value < 0.05, **High level of significance at P-value < 0.01.

Table 9 Correlation Between Serum Ferritin and Hepatic and Cardiac MRI T2*

		Liver MRI T2*	Cardiac MRI T2*
Ferritin	N	200	200
	Pearson Correlation	-0.681**	-0.284**
	Significance (2-tailed)	0.000	0.002

Notes: *Modified technique where MRI signal decay rates are proportional to liver or cardiac iron concentration, **Statistically significant at P-value < 0.01.

done by Royal Australasian College of Physicians, the frequency of DM among patients' age >18 years was (2/29) 6.89%, (sample size 29 patients).²⁷ Also, this latter result was very close to our results. Regarding the assessment of ferritin level, we found a statistical significance for serum ferritin level among the patients with DM, and those patients need proper iron chelation protocol,²⁸⁻³⁴ in combination with DM treatments. When making a comparative study with previous reports, we observed that serum ferritin was associated with DM in BTM patients.^{4-6,19}

In the Italian work, the mean serum ferritin level associated with DM was (3790 ±2566 ng/mL), P < 0.05. Our result was in agreement with that work where the mean serum ferritin was 3909.0200 ±1935.72465 ng/mL; P-value = 0.014. In a previous work,^{12,23} the ferritin level was greater than 2500 ng/mL, P < 0.001.

Concerning the evaluation of BMI with the development of diabetes mellitus, we found out that BMI was associated with DM (P < 0.001). This result was in the same direction of the previous work where BMI was statistically significant with DM,¹² P= 0.046.

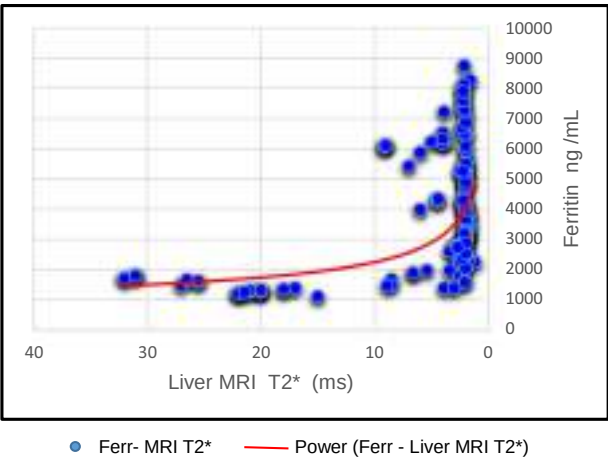


Figure 6 Correlation between liver MRI T2* and ferritin.
Note: The red curve is the trend-line of type (power) to fit the data.

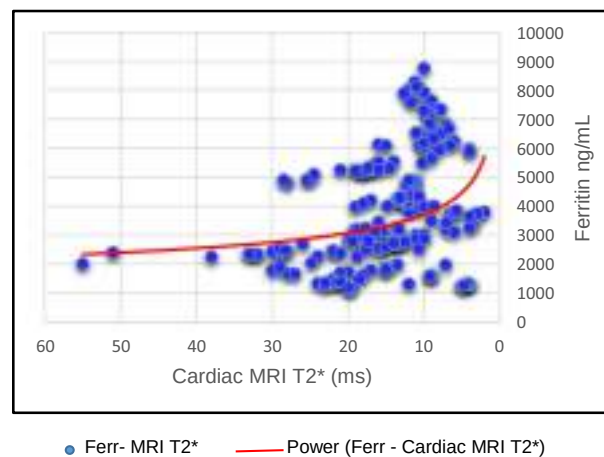


Figure 7 Correlation between cardiac MRI T2* and ferritin.

Note: The red curve is the trend-line of type (power) to fit the data.

Moreover, in the current study, we found that gender was not statistically significant with DM where $P=0.153$. This result agreed with the result of a previous research,¹² where gender had no significance with DM; $P=0.129$. Also, in the work done by the Italian group, gender was not associated with DM.^{12,23}

Some papers indicated that T2* Magnetic Resonance Imaging (MRI) is a sensitive method for detecting iron content in body organs. The cardiac T2* MRI is considered as predictors of diabetes,^{35,36} and it is correlated with the pancreatic values of T2*.

In the current work, a significant moderate negative correlation between liver MRI T2* and serum ferritin level was observed. This means that the serum ferritin level is predictive of liver iron level. Other work had shown a significant association between ferritin level and iron level of liver MRI T2*.⁴⁰ Another study exhibited that serum ferritin levels had moderate negative correlation with liver MRI T2* values ($r = -0.586$, $P < 0.0001$).⁴¹

In another research using hepatic MRI R2 method (FerriScan®) with sample size ($N=24$) and age ranges 9–35 years old, the liver iron concentrations (LIC) had significant moderate correlation with serum ferritin levels ($r = 0.512$; $p = 0.011$).⁴² In the current study with sample size ($N=200$) and ages ≥ 18 years), there was a moderately negative correlation, which had statistical significance between liver MRI T2* and serum ferritin readings ($r = -0.681$, $P < 0.001$). Although this method was different from the previous one (LIC using MRI R2 method; FerriScan®), they had similar prediction that both techniques indicated significant moderate correlation with serum ferritin levels.

In the current study, a poor correlation between cardiac MRI T2* and serum ferritin level was observed. A recent study used cardiovascular magnetic resonance with T2-weighted sequences (CMR T2*) for thalassemia major patients, to determine the association between (CMR T2*) and serum ferritin in thalassemia major patients. It proved that no

Table 10 Bivariate Correlations Analysis of Liver and Cardiac MRI T2*

		Liver MRI T2*	Cardiac MRI T2*
Liver MRI T2*	Pearson Correlation	1	0.064
	Significance (2-tailed)		0.367
	N	200	200
Cardiac MRI T2*	Pearson Correlation	0.064	1
	Significance (2-tailed)	0.367	
	N	200	200

Notes: *Modified technique where MRI signal decay rates are proportional to liver or cardiac iron concentration.

significant correlation was found between CMR T2* and the serum ferritin ($P = 0.158$, $r = 0.201$).⁴³ Another work showed that no correlation was found between serum ferritin and cardiac iron levels using MRI T2* in BTM patients.⁴⁴

Moreover, our study proved that there was very poor correlation between cardiac and liver MRI T2* readings among BTM patients with no statistical significance ($r=0.064$, $p=0.367$). A work with sample size ($N = 180$) has showed significant but very poor correlation between liver MRI T2* and cardiac MRI T2* ($r = 0.18$, $P < 0.05$).⁴⁵

Based on the above discussion, since there was a significant correlation between serum ferritin and liver MRI T2*, serum ferritin can be used for evaluating liver iron levels for economic reasons. But cardiac MRI T2* is preferred for accurate measuring cardiac iron levels instead of using serum ferritin levels.

Comparative Study of Distribution of DM with Previous Studies

In this section, comparative study of distribution of DM with previous studies. This is given in (Table 11). The overall prevalence of DM among our BTM patients was 6.5%. The endocrinopathy was studied with the available and suggested parameters model. Recently, a work was done by a multicenter clinic revealed that the prevalence of DM among TDT patients (transfusion-dependent thalassemia) with sample size 713 patients was 15.8%. The parameters including age, ICT and splenectomy were studied in the research.¹²

Some researchers reported that the age and duration of blood transfusion were significantly associated with DM, while others stated that the prevalence of DM was 8.9% among 56 BTM patients, and neither serum ferritin nor gender was associated with that endocrine disorder.¹³ This may be due to small sample size.

Table 11 Comparative Study of Our Work with Previous Studies

DM in Current and Previous Works	Sample Size N	% Prevalence	Correlated Parameters With DM	Status
DM (in current work)	200	6.5	Age groups	(Correlated)
			(27-< 32) years old	Most prevalent
			Ferritin	(Correlated)
			Level >2500 ng/mL	(Critical)
			BMI	(Correlated)
			Hb	(Not correlated)
			Gender	(Not correlated)
DM in work ¹²	713	15.8	Age	(Correlated)
			Ferritin	(Correlated)
			Splenectomy	(Correlated)
			ICT	(Not Correlated)
			Gender	(Not Correlated)
DM in work ¹³	56	8.9	Ferritin	(Not Correlated)
			Gender	(Not Correlated)
DM in work ¹⁵	64	4.7	Ferritin (Not correlated)	(Not correlated)
DM in work ¹⁷	267	10	Ferritin (Correlated)	(Correlated)
DM in work ¹⁸	148	8.8	Age (Correlated)	(Correlated)
			Splenectomy	(Correlated)
			Ferritin (Correlated)	(Correlated)

In another work, serum ferritin and hemoglobin levels are related to IGT (impaired glucose tolerance).¹⁴ The work done in previous studies^{15,16} revealed that the prevalence of DM was 4.7% among sample size of 64 patients, and their results showed that the serum ferritin was not correlated. It is worth noting that, in the previous mentioned works, there were different variables, eg, ferritin, that truly correlated with the endocrinopathy such as DM. However, serum ferritin is of critical importance and leads to damage the endocrine glands and the BTM patients should be immediately medically treated using iron chelation therapy.

Conclusion

The overall frequency of Diabetes mellitus among the BTM patients was 6.5%. The maximum occurrence of this endocrine dysfunction was 4.5% that associated with ferritin level range (>3500 ng/mL). The minimum serum ferritin level was 2501 ng/mL.

It was found that there was an association between serum ferritin level and that endocrine dysfunction ($P = 0.014$). The serum ferritin level 2501 ng/mL can be considered as a risk factor of that endocrinopathy. The maximum frequency of that endocrinopathy was found in the BTM patients with BMI of class “overweight”. Since BMI had significant association with DM, then factors such as diet and physical activity should be advised.

In a few words, DM was found among BTM patients and the following critical points should be taken into considerations: (a) Age group (27–<32) years old and severe serum ferritin >2500 ng/mL should be properly managed and treated immediately to stop or reduce that endocrine complication. Proper ICT protocol must be used especially to those BTM patients. (b) More research should be done to find out the correlation of different parameters with DM. This requires longitudinal observations of the BTM patients taking ICT and different managements.

Serum ferritin can be used for estimating liver iron levels for economic factors. It is preferred to perform cardiac MRI T2* for measuring cardiac iron level instead of using serum ferritin level. It is important to evaluate precisely the iron level in both the liver and heart for tailoring optimal chelation treatment protocol to avoid or minimize mortality in BTM patients. Based on the above results, it is recommended to do scanning for cardiac MRI T2* every six months in severe cases and annually for moderate cases.

Abbreviations

S.Phosph, serum phosphorous; Hb, hemoglobin; ALT, aminotransferase alanine; AST, aminotransferase aspartate; Cr, creatinine; n, number; Std, standard; BTM, beta thalassemia major; BMI, body mass index; Sig, significance; Ferr, ferritin; MRI, magnetic resonance image.

Data Sharing Statement

No data sharing as this manuscript and the data were not published elsewhere.

Ethical Approval

This work complies with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB) Faculty of Medicine, Zagazig University, Egypt (Approval IRB #: 5895- 8-3-2020). The data are accessed confidentially by the authors. The information was not specific to certain patients and the work was cross-sectional study. The patient informed consent was waived for this research.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

- Gamberini MR, Sanctis DV, Gilli G. Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassemia major followed from 1980 to 2007 in the Ferrara Centre. *Ped Endocrinol Rev.* 2008;6(1):158–169.
- Maria IA, Dimitrios NK, Loukas A, et al. Liver, bone marrow, pancreas and pituitary gland iron overload in young and adult thalassemic patients: a T2 relaxometry study. *Eur Radiol.* 2007;17:3025–3030. doi:10.1007/s00330-007-0683-1
- Papakonstantinou O, Ladis V, Kostaridou S, et al. The pancreas in beta -thalassemia major: MR imaging features and correlation with iron stores and glucose disturbances. *Eur Radiol.* 2007;6:1535–1543. doi:10.1007/s00330-006-0507-8
- Palak YT, Neelaba KM, Chandrika GA. Assessment of iron overload in beta thalassemia major patients by serum ferritin level. *Eur J Mol Clin Med.* 2022;9:828–832.
- Farmaki K, Angelopoulos N, Anagnostopoulos G, et al. Effect of enhanced iron chelation therapy on glucose metabolism in patients with beta-thalassaemia major. *Br J Haematol.* 2006;134:438–444. doi:10.1111/j.1365-2141.2006.06203.x
- Chatterjee R, Bajoria R. New concept in natural history and management of diabetes mellitus in thalassemia major. *Hemoglobin.* 2009;33:S127–S130. doi:10.3109/09553000903347880
- Messina MF, Lombardo F, Meo A, et al. Three-year prospective evaluation of glucose tolerance, beta-cell function and peripheral insulin sensitivity in non-diabetic patients with thalassemia major. *J Endocrinol Invest.* 2002;25:497–501. doi:10.1007/BF03345490
- Platis O, Anagnostopoulos G, Farmaki K, et al. Glucose metabolism disorders improvement in patients with thalassemia major after 24–36 months of intensive chelation therapy. *Pediatr Endocrinol Rev.* 2004;2:279–281.
- Karuppiah D. Evaluation and management of diabetes in patients with thalassemia major. *J Ceylon College Phys.* 2015;46:39–41. doi:10.4038/jccp.v46i1-2.7749
- American diabetes association ADA. Introduction: standards of medical care in diabetes. *Diabetes Care.* 2018;2018(Suppl 1):S1–S2. doi:10.2337/dc18-Sint01
- Frank QN. Body mass index: obesity, BMI, and health: a critical review. *Nutr Today.* 2015;3:117–128. doi:10.1097/NT.000000000000092
- Bordbar M, Bozorgi H, Saki F, et al. Prevalence of endocrine disorders and their associated factors in transfusion dependent thalassemia patients: a historical cohort study in southern Iran. *J Endocrinol Invest.* 2019;42:1467–1476. doi:10.1007/s40618-019-01072-z
- Mowla A, Karimi M, Afrasiabi A, et al. Prevalence of diabetes mellitus and impaired glucose tolerance in beta-thalassemia patients with and without hepatitis C virus infection. *Pediatr Endocrinol Rev.* 2004;2(Suppl 2):282–284.
- Gamberini MR, Fortini M, Sanctis DV, et al. Diabetes mellitus and impaired glucose tolerance in thalassaemia major: incidence, prevalence, risk factors and survival in patients followed in the Ferrara Center. *Pediatr Endocrinol Rev.* 2004;2(Suppl 2):285–291.
- Sharma A, Seth A, Chandra J, et al. Endocrinopathies in adolescents with thalassaemia major receiving oral iron chelation therapy. *Paediatr Int Child Health.* 2016;1:22–27. doi:10.1179/2046905514Y.0000000160
- Sharma A, Arora E, Singh H. Hypersensitivity reaction with deferasirox. *J Pharmacol Pharmacother.* 2015;2:105–106. doi:10.4103/0976-500X.155491
- Thuret I, Ponderre C, Loundou A, et al. Complications and treatment of patients with thalassemia in France: results of the National Registry. *Haematologica.* 2010;5:724–729. doi:10.3324/haematol.2009.018051
- Ali B, Javad S, Daryoush R, et al. Diabetes mellitus in Thalassemia major patients: a report from the Southeast of Iran. *J Clin Diagn Res.* 2017;11(5):BC01–BC04. doi:10.7860/JCDR/2017/24762.9806
- De Assis RA, Ribeiro AA, Kay FU, et al. Pancreatic iron stores assessed by magnetic resonance imaging (MRI) in beta thalassemic patients. *Eur J Radiol.* 2012;81:1465–1470. doi:10.1016/j.ejrad.2011.03.077
- Suvarna J, Ingle H, Deshmukh CT. Insulin resistance and beta cell function in chronically transfused patients of thalassemia major. *Indian Pediatr.* 2006;43:393–400.
- Lu MY, Peng SS, Chang HH, et al. Cardiac iron measurement and iron chelation therapy in patients with beta thalassemia major: experience from Taiwan. *Transfus Med.* 2013;23:100–107. doi:10.1111/tme.12014
- Noetzli LJ, Papudesi J, Coates TD, et al. Pancreatic iron loading predicts cardiac iron loading in thalassemia major. *Blood.* 2009;114:4021–4026. doi:10.1182/blood-2009-06-225615225615
- Italian Working Group on Endocrine Complications in Non-endocrine Diseases 1995. Multicenter study on prevalence of endocrine complications in thalassemia major. *Clin Endocrinol.* 1995;42:581–586. doi:10.1111/j.1365-2265.1995.tb02683.x
- Nolan JJ, Faerch K. Estimating insulin sensitivity and beta cell function: perspectives from the modern pandemics of obesity and type 2 diabetes. *Diabetologia.* 2012;55:2863–2867. doi:10.1007/s00125-012-2684-0
- Modell B, Khan M, Darlison M. Survival in beta-thalassemia major in the UK: data from the UK Thalassemia register. *Lancet.* 2000;355(9220):2051–2052. doi:10.1016/S0140-6736(00)02357-6
- Fedele B, Rosa C, Paola B, et al. Complications pattern and burden of the disease in patients affected by beta-thalassemia major. *Curr Med Res Opin.* 2017;55:1525–1533. doi:10.1080/03007995.2017.1326890
- Nimalie J, Perera NS, Mathews S, et al. Overview of endocrinopathies associated with beta-thalassemia major. *Intern Med J.* 2010;40:689–696. doi:10.1111/j.1445-5994.2010.02254.x
- Kontoghiorghes GJ. A new era in iron chelation therapy: the design of optimal, individually adjusted iron chelation therapies for the complete removal of iron overload in thalassemia and other chronically transfused patients. *Hemoglobin.* 2009;33:332–338. doi:10.3109/03630260903217182
- Hoffbrand AV, Gorman A, Laulicht M, et al. Improvement in iron status and liver function in patients with transfusional iron overload with long-term subcutaneous desferrioxamine. *Lancet.* 1979;1(8123):947–949. doi:10.1016/s0140-6736(79)91721-5
- Kontoghiorghes GJ, Aldouri MA, Hoffbrand VJ, et al. Effective chelation of iron in beta thalassemia with the oral chelator 1, 2-dimethyl-3-hydroxypyrid-4-one. *Br Med J.* 1987;295:1509–1512. doi:10.2307/29529126
- Kolnagou A, Economides E, Kontoghiorghes GJ, Kontoghiorghes GJ. Long term comparative studies in thalassemia patients treated with deferoxamine or deferoxamine/deferiprone combination. Identification of effective chelation therapy protocols. *Hemoglobin.* 2008;32:41–47. doi:10.1080/03630260701727085

32. Porter JB, Elalfy MS, Taher Y, et al. Efficacy and safety of deferasirox at low and high iron burdens: results from the EPIC magnetic resonance imaging sub-study. *Ann Hematol*. 2013;92:211–219. doi:10.1007/s00277-012-1588-x
33. Kolnagou A, Kleanthous M, Kontoghiorghes GJ. Reduction of body iron stores to normal range levels in thalassemia by using a deferiprone/deferrioxamine combination and their maintenance thereafter by deferiprone monotherapy. *Eur J Haematol*. 2010;85:430–438. doi:10.1111/j.1600-0609.2010.01499.x
34. Farmaki KI, Tzoumari C, Pappa G, Berdoukas V, Berdoukas V. Normalization of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassemia major. *Br J Haematol*. 2010;148:466–475. doi:10.1111/j.1365-2141.2009.07970.x
35. Kolnagou A, Yazman D, Economides C, Eracleous E, Kontoghiorghes GJ. Uses and limitations of serum ferritin, magnetic resonance imaging T2 and T2* in the diagnosis of iron overload and in the ferritkinetics of normalization of the iron stores in thalassemia using the International Committee on Chelation deferiprone/deferrioxamine combination protocol. *Hemoglobin*. 2009;33:312–322. doi:10.3109/03630260903213231
36. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star T2*. Magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J*. 2001;22:2171–2179. doi:10.1053/euhj.2001.2822
37. Pennell DJ. T2* magnetic resonance and myocardial iron in Thalassemia. *Ann NY Acad Sci*. 2005;1054:373–378. doi:10.1196/annals.1345.045
38. Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation*. 2009;120:1961–1968. doi:10.1161/CIRCULATIONAHA.109.874487
39. Roghi A, Maria DC, John CW, et al. Absence of cardiac siderosis despite hepatic iron overload in Italian patients with thalassemia intermedia: an MRI T2* study. *Ann Hematol*. 2010;89:585–589. doi:10.1007/s00277-009-0879-3
40. Voskaridou E, Douskou M, Terpos E, et al. Magnetic resonance imaging in the evaluation of iron overload in patients with beta thalassemia and sickle cell disease. *Br J Haematol*. 2004;126(5):736–742. doi:10.1111/j.1365-2141.2004.05104.x
41. Zamani F, Razmjou S, Akhlaghpour S, et al. T2* magnetic resonance imaging of the liver in thalassemic patients in Iran. *World J Gastroenterol*. 2011;17(4):522–525. doi:10.3748/wjg.v17.i4.522
42. Ibrahim K, Sandra A, Prem C, et al. Severe liver iron concentrations (LIC) in 24 patients with β -thalassemia major: correlations with serum ferritin, liver enzymes and endocrine complications. *Mediterranean J Hematol Infectious Dis*. 2018;10(1):1–8. doi:10.4084/MJHID.2018.062
43. Reihaneh ZR, Moien H, Mohaddeseh B, Dalir Y. T2-weighted cardiovascular magnetic resonance and echocardiographic arterial elasticity criteria for monitoring cardiac siderosis in patients with beta-thalassemia major. *Res Med Sci*. 2022;27:1–5. doi:10.4103/jrms.jrms_705_21
44. Tushar MK, Viraj PS, Sameeh UZ, et al. The role of cardiac T2* magnetic resonance imaging in the assessment of myocardial iron concentration in patients with beta-thalassemia major. *Asian J Med Sci*. 2022;13:79–84. doi:10.3126/ajms.v13i6.43049
45. Au WY, Lam WW, Chu WW, et al. A cross-sectional magnetic resonance imaging assessment of organ specific hemosiderosis in 180 thalassemia major patients in Hong Kong. *Haematologica*. 2008;93:784–786. doi:10.3324/haematol.12367

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Anemia Among Women Using Family Planning at Public Health Facilities in Ambo Town, Central Ethiopia: Multi-Center Cross-Sectional Study

Shalama Lekasa Nagari, Gudina Egata, Ame Mehadi, Tahir Ahmed Hassen, Temam Beshir Raru, Mohammed Abdurke, Mohammed Yuya, Shemsedin Abdulkadir, Hiwot Berhanu & Kedir Teji Roba

To cite this article: Shalama Lekasa Nagari, Gudina Egata, Ame Mehadi, Tahir Ahmed Hassen, Temam Beshir Raru, Mohammed Abdurke, Mohammed Yuya, Shemsedin Abdulkadir, Hiwot Berhanu & Kedir Teji Roba (2023) Anemia Among Women Using Family Planning at Public Health Facilities in Ambo Town, Central Ethiopia: Multi-Center Cross-Sectional Study, Journal of Blood Medicine, , 83-97, DOI: [10.2147/JBM.S400191](https://doi.org/10.2147/JBM.S400191)

To link to this article: <https://doi.org/10.2147/JBM.S400191>



© 2023 Nagari et al.



Published online: 08 Feb 2023.



Submit your article to this journal



Article views: 457



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Anemia Among Women Using Family Planning at Public Health Facilities in Ambo Town, Central Ethiopia: Multi-Center Cross-Sectional Study

Shalama Lekasa Nagari¹, Gudina Egata², Ame Mehadi³, Tahir Ahmed Hassen³, Temam Beshir Raru³, Mohammed Abdurke³, Mohammed Yuya³, Shemsedin Abdulkadir⁴, Hiwot Berhanu³, Kedir Teji Roba³

¹College of Health Sciences, Assosa University, Assosa, Ethiopia; ²College of Health and Medical Sciences, Addis Ababa University, Addis Ababa, Ethiopia; ³College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ⁴College of Health and Medical Sciences, Arsi University, Asella, Oromia, Ethiopia

Correspondence: Ame Mehadi, School of Nursing and Midwifery, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia, Tel +251919118839, Fax +251256668081, Email mehadiame1554@gmail.com

Background: Anemia affects more than a quarter of non-pregnant women over the globe, with Sub-Saharan Africa bearing a disproportionate share. Although the use of family planning is beneficial in reducing anemia, lack of scientific study on anemia among family planning users of reproductive-age women is notable, particularly in the study setting. The purpose of this study was to determine the extent of anemia and associated factors in women who used family planning.

Methods: A cross-sectional multi-centered study was conducted from March 3 to 29, 2019, among 443 non-pregnant reproductive age (15 to 49 years) women receiving family planning services in Ambo town. Sample size was calculated using Epi-info version 7 software. Participants were selected by systematic random sampling technique. Trained data collectors collected data using a structured pretested questionnaire, as well as venous blood and stool samples. Epi-Data and SPSS were used to enter and analyze data. The effect of independent variables on the outcome variable was determined by binary logistic regression analysis with adjusted odds ratio at 95% confidence interval and 5% margin of error. P-value <0.05 was used to declare statistical significance.

Results: This study revealed 28% (95% CI:23.9%, 32.3%) magnitude of anemia. Age of 25–35 years [AOR:2.84, 95% CI:1.74, 4.64], implantable family planning method [AOR: 0.34, 95% CI: 0.12, 0.96], no previous use of family planning [AOR:2.62, 95% CI: 1.62, 4.24], household food insecurity [AOR: 2.04, 95% CI: 1.06, 3.93], parasite infestations [AOR:2.01, 95% CI: 1.12, 3.63], and regular intake of coffee/tea within 30 minutes post meal [AOR:3.85, 95% CI:1.24, 11.92] were independently associated with anemia.

Conclusion: Anemia is a moderate public health concern among reproductive-age women receiving family planning services in the study area. There are missed opportunities to address the anemia burden during family planning services. This study emphasizes the importance of nutritional screening for early detection and targeted interventions for healthcare workers in reducing missed opportunities to prevent and control anemia in vulnerable populations.

Keywords: anemia, family planning, reproductive age women, Ambo, Ethiopia

Introduction

Anemia is a worldwide public health concern and a major challenging problem in resource-limited countries.^{1,2} Over one-third of the world population is deficient in single or multiple micronutrients mainly due to inadequate intake.^{3–5} More than 2.2 billion of the world's population are affected by anemia at a rate of 33%.⁶ Pregnant and non-pregnant women of reproductive age (WRA) (15–49 years) and children compose the population group most affected by anemia.⁷ It affects 273 million (43%) world children, 496 million (30%) of WRA, and 32 million (38%) pregnant women over the globe.^{6,8,9} This burden is disproportionately concentrated in low socioeconomic groups.⁹

Anemia is considered unacceptable in any population group or subgroup when the overall prevalence exceeds 5%, which is mostly reflected in LMICs.¹⁰ Across LMICs, a great variation in the overall prevalence of anemia in WRA is notable. The

highest prevalence was reported from countries in the Middle East, South Asia and West African regions in 2018.¹¹ Countries like Yemen (57.4%), India (49.9%), Gambia (50.3%), Senegal (47.3%) and Mali (47.6%) were among the others with high prevalence rates of anemia.¹¹ Ethiopia is one of the countries with the highest rates of anemia in Africa.¹¹ In Ethiopia, the rate of anemia prevalence in WRA was reported to be 23.4% in 2016, nearly half the rate reported in 1990 (48%).^{12,13}

Anemia prevalence among NPWs ranges from 37.7% to 41.5% across the WHO regions of South-East Asia, the Eastern Mediterranean, and Africa.¹⁴ Iron deficiency contributes to more than half of the global anemia burden and is caused by insufficient intake, increased requirements, and chronic blood loss.¹⁴ Under nutrition in pregnant women can set in motion and perpetuate a cycle of intergenerational malnutrition, infection, and underdevelopment.⁵ This in turn has an impact on the individual and population's productivity and economic potential.⁵ Anemia during pregnancy is linked to poor birth outcomes such as maternal and neonatal mortality, as well as impaired and delayed cognitive development in children.^{15,16} In addition, increased risk of infections, impaired cognitive and physical development as well as poor school performance among offspring are associated with anemia.¹⁷

Failing to invest in interventions to reduce anemia among NPWs is estimated to result in 265 million more cases of anemia in women in 2025 than in 2015, as well as nearly 800,000 more child deaths and over 7000–14,000 more maternal deaths.¹⁸ In addition to negative health consequences, anemia's economic impact on human capital results in annual losses of billions of dollars.⁹ Investing in anemia prevention, on the other hand, could increase the economic productivity of LMIC by \$110 billion. Economic returns on investment in this set of interventions are estimated to be \$12 for every \$1 invested.¹⁸

Nutrition, socioeconomic status, health care seeking practice, poor dietary practice, hygiene and sanitary practice, Obstetric and gynecologic factors, health condition, type of family planning used, and women's BMI are all factors known to contribute to the problem.^{2,19–26} This problem is exacerbated in Ethiopia by pre-existing food insecurity.²⁷ Unequal household food allocation can expose WRA to anemia because they may lack access to iron-rich foods, particularly in areas where a culture favors older boys, men, and elders, and bias in household food allocation.^{5,27} The type of women's diet was also found to influence their risk of developing anemia. In one study, the risk of developing anemia was higher among vegetarians (64.9%) than mixed diet consumers (49.5%) which might be due to low intake of heme iron.²⁸

The use of family planning has been shown to help with anemia. This could be by extending or lengthening the intervals and inhibiting pregnancies. Although hemoglobin levels vary depending on the contraceptive method used, users of any contraceptive method had higher hemoglobin levels than non-users.^{22,29,30} Studies conducted so far in Ethiopia have shown that non-users of contraceptive methods have a higher prevalence of anemia than contraceptive users WRA.³⁰ A cross-sectional community-based study conducted in four major regions of Ethiopia found that women who used no family-planning methods had a slightly higher prevalence of anemia (37.4%) than their counterparts.²⁵

Using contraceptive methods is associated with a lower risk of anemia. In one study, anemia was 1.38 times more likely among non-users of contraception (21.7%) compared to contraceptive users (11.6%).³¹ Analytic study of the 2016 EDHS data revealed that the likelihood of developing anemia was 1.5 times ($p=0.02$) higher among non-pregnant WRA who did not use contraceptive methods than those who used contraception.³² In other studies, not using contraceptive methods was found to be an independent factor associated with anemia in NPW of reproductive age.^{22,26}

Despite this benefit, a significant scarcity of scientific studies and data that estimate the magnitude of anemia and associated risk factors among family planning users NPW of reproductive age is notable. Thus, the aim of this study was to determine the extent of anemia and its potential risk factors associated among family planning users NPW of reproductive age in Ambo town, thereby making family planning service a window of opportunity.

Methods and Materials

Study Period and Setting

This study was carried out in central Ethiopia in Ambo town public health institutions. Ambo town is the capital of the West Shewa zone, one of the zones in the regional state of Oromia, and is found 114 kilometers from Addis Ababa (the country's capital city) in the western direction. The town has six kebeles of which three are rural and three are urban kebeles (the last unit of administration in Ethiopia). The town's total population was 108,000 as of 2018 with 53,400

males and 54,600 females. In Ambo Town, there are thirteen public health institutions of which two are hospitals (1 referral and 1 general), two are health centers and nine are health posts. Twenty-one private clinics are also available in the town.^{33,34} The study was carried out from March 3 to 29, 2019 in each two public health centers and hospitals.

Populations and Design

A Multicenter institutional-based cross-sectional study design was used among reproductive age (15 to 49 years) women receiving family planning services in Ambo town. All reproductive-age women receiving family planning services in health facilities of the general public in Ambo town were the source population of the current study whereas reproductive-age women receiving family planning services during the study period and fulfilling the inclusion criteria were the study population. However, those women who came for family planning services for the first time, had a history of abortion in the previous three months, gave birth in the previous three months, had a blood transfusion in the previous three months, and were on anemia treatments were excluded.

Sample Size and Sampling Methods

The sample size was calculated using Epi-info version 7 software with a 95% confidence interval, 80% power, and a 1:1 ratio of unexposed to exposed groups based on the type of family planning method used. Available revealed that the proportions of exposed (Barrier methods) and unexposed (Implant) were 41.0% and 27.7%, respectively.³⁵ This results in a sample of 428. Adding a 10% non-response rate yielded a sample of 471. Finally, 471 participants were recruited for this study. However, data were gathered from 443 women who attended family planning clinics.

Measurements and Definitions

Socioeconomic and demographic characteristics (sex, age, educational and literacy status, presence of under-five children in the household, residence, occupation, household income), housing conditions (number of family members in the household, and household sanitation), nutrition and food-related conditions (food security and dietary diversity status), and women's health conditions (menstruation, pregnancies, history of miscarriages, and body mass index) were independent variables.

Body mass index- is a simple weight-to-height index that is age-independent for adult populations and is the same for both genders. Weight in kilograms divided by height in meters squared (kg/m^2) is calculated and classified as follows: $<18.5 \text{ kg}/\text{m}^2$ indicates underweight, $18.5\text{--}24.9 \text{ kg}/\text{m}^2$ indicates normal weight, $25.0\text{--}29.9 \text{ kg}/\text{m}^2$ indicates overweight, and $30.0 \text{ kg}/\text{m}^2$ indicates obesity.¹⁵

Data Collection Methods and Procedures

Socio-demographic, obstetric/gynecologic, food and nutritional, anthropometric measurements, and health-related clinical data were collected by trained clinical nurses using a structured and pretested interviewer-administered questionnaire developed after a review of various pieces of literature,^{22,37} while four experienced and trained laboratory technicians collected laboratory data. Data were collected in the local language (Afan Oromo) version of data collection tool. Women's dietary diversity score (WDDS) data were collected using a 24 hrs dietary diversity questionnaire and were analyzed by calculating women's dietary diversity score from 10 food groups proposed by FANTA to calculate dietary diversity score for women.^{35–37} Women who scored five or more were classified as having high dietary diversity whereas those scored lower than five were classified as low dietary diversity.³⁷ Household food security data were collected by the HFIAS tool developed by FANTA.^{35,37–40}

Four milliliters of venous blood samples were collected from each study participant. Samples collected from both health centers (Ambo and Awaro) using 4mL of K3 EDTA (ethylenediamine-tetraacetic acid) tube were taken to Ambo general hospital within four hours of collection for analysis.^{12,14,41,42} Trained laboratory technicians analyzed the samples. Hemoglobin concentration was measured using CELL-DYN 1800® (Abott, USA),^{12,14,41,42} and it was adjusted only for altitude since there was no smoking reported. Hemoglobin concentration adjustment for altitude was done to subtract the adjustment from the measured Hb concentration at the relevant altitude above sea level to get the sea-level value.^{10,42–44} Then anemia was diagnosed based on hemoglobin concentration ($\text{Hb} <$

12g/dL) for non-pregnant adult women.^{8,41,45} Finally, women with a hemoglobin concentration less than 12g/dl were classified as anemic and those with 12g/dl and above were classified as non-anemic.⁸

Five grams of stool sample was collected from each study participant for parasitological data using clean, wide-mouthed, and leak-proof stool cups. The stool sample was then examined by wet mount preparation within 10–15 minutes of collection. To determine the presence of parasites in the stool, leftover samples were processed for the formal-ether concentration technique.²²

Data Quality Management

A structured and pretested interviewer-administered questionnaire which was developed after a review of various pieces of literature,^{22,37} was used to collect data. The tool was initially prepared in English and then translated into Afan Oromo's local language, then translated back to English by language experts to check for language consistency, and the Afan Oromo version questionnaires were used for data collection. The data collection tool was pre-tested on 5% of the sample size and necessary amendments were done based on the results before actual data collection. Data collectors were four clinical nurses and four laboratory technicians and they received one-day training on the study's objectives, data collection methods, and tools. Two supervisors and a principal investigator closely supervised the data collection process. Experienced and trained laboratory technicians collected laboratory-related data and carried out all activities following the manufacturer's instructions and specific standard operating procedures. The expiry dates of all reagents and quality control materials were checked. Laboratory results were documented using standard reporting formats and unique identification numbers. The collected data were rechecked daily at the end of each day to ensure completeness and consistency.

Data Analysis and Processing

All checked data were coded, entered into Epi-Data version 3.1, cleaned, and exported to SPSS (version 23) for analysis. Data were summarized using descriptive statistics such as frequencies and percentages. Variables with p-values less than 0.20 in the bivariable logistic regression analysis were considered for multivariable logistic regression analysis. The logistic regression model's fitness was determined using Hosmer-Lemeshow, and it was declared statistically insignificant.

Results

Socio-Demographic Characteristics

Four hundred seventy-one (471) reproductive-age women who received family planning services were considered for this study and 443 women participated in the survey with a 94.05% response rate. The majority of study participants, 415 (93.7%), were under the age of 35 (Table 1).

Obstetric, Gynecologic, and Reproductive Characteristics

The majority of women, 249 (56.2%), used injectable contraception, whereas IUCD was used by only 23 (5.2%), and two-thirds, 147 (66.8%), had a birth interval of more than two years (Table 2).

Nutrition and Dietary Characteristics

More than half of the participants, 241 (54.4%), had consumed any form of grain-based meal on a daily basis in the seven days preceding data collection, while vegetables, fruits, and animal sources were consumed less frequently. Almost all of the 417 respondents (94.1%) said they had enough food diversity. The vast majority (389, or 87.8%) stated that they come from food-secure homes (Table 3).

Health and Lifestyle-Related Characteristics

The majority of study participants, 407 (91.9%), were free of sickness for at least two weeks previous to data collection, whereas 66 (14.9%) had one or more intestinal parasites. *G.lambia* (28.4%), *H.worm* (23.9%), *A.lumbricoids* (22.4%), and *E.histolytica* (16.4%) were the most prevalent parasites detected (Table 4).

Table I Socio-Demographic Characteristics of Women Receiving Family Planning Service in Public Health Facilities in Ambo Town, Central Ethiopia, 2019 (n=443)

Variables	Category	Frequency	Percentage
Age of the women	15–24	199	44.9
	25–35	216	48.8
	36–49	28	6.3
Religion	Orthodox	139	31.4
	Muslims	24	5.4
	Protestant	273	61.6
	Wakefata	7	1.6
Marital status	Married	406	91.6
	Divorced	9	2.0
	Widowed	8	1.8
	Single	14	3.2
	Separated	6	1.4
Women's educational status	No formal education	123	27.8
	Primary education	146	33.0
	Secondary and above	174	39.3
Women's occupational status	House maker	279	63.0
	Government employee	78	17.6
	Merchant	42	9.5
	Daily laborer	44	9.9
Husband's educational status	No formal education	89	21.9
	Primary education	104	25.6
	Secondary and above	213	52.5
Husband's occupational status	Farmer	72	17.7
	Government employee	146	36.0
	Merchant	66	16.3
	Daily laborer	91	22.4
	Driver	31	7.6
Family income(ETB)	<1000	94	21.2
	1000 and above	349	78.8
Family size	<5	360	81.3
	>5	83	18.7

(Continued)

Table 1 (Continued).

Variables	Category	Frequency	Percentage
Under five children in the HH	None	87	19.6
	1	308	69.5
	2 and above	48	10.8
Under two children in the HH	Yes	270	60.1
	No	173	39.9
Source of drinking water	Protected	402	90.7
	Unprotected	41	9.3
ITN use during night	Yes	224	50.6
	No	219	49.4
Toilet facility	Yes	413	93.2
	No	30	6.8

Abbreviations: HH, Households; ITN, Insect treated nets.

Table 2 Obstetric and Gynecologic Characteristics of Women Receiving Family Planning Service in Public Health Facilities in Ambo Town, Central Ethiopia, 2019 (n=443)

Variables	Category	Frequency	Percentage
Duration of menses in days	≤5	393	88.7
	>5	50	11.3
Number of sanitary pads used daily during menses	≤2	315	71.1
	>2	128	28.9
Parity	None	56	12.6
	1–2	249	56.2
	3–5	120	27.1
	>5	18	4.1
Number of live births	≤2	266	68.7
	3–5	108	27.9
	>5	13	3.4
Place of delivery	Home	45	11.6
	Institutional	342	88.4
Birth spacing in years	≤2	73	33.2
	>2	147	66.8
Breast feeding	Yes	228	58.9
	No	159	41.1

(Continued)

Table 2 (Continued).

Variables	Category	Frequency	Percentage
Duration of breast feeding	3–11 months	117	51.3
	12–23 months	107	46.9
	24 months and above	4	1.7
ANC follow up	Yes	349	90.2
	No	38	9.8
Got IFA supplementation	Yes	341	97.7
	No	8	2.3
Type of FP being used	OCP	66	14.9
	Injectable	249	56.2
	Implantable	105	23.7
	IUCD	23	5.2
Any FP used previously	Yes	296	66.8
	No	147	33.2

Abbreviations: ANC, Antenatal care; FP, Family planning; IFA, Iron-folic acid; FP, Family planning; OCP, Oral Contraceptive Pill; IUCD, Intra-Uterine Contraceptive Device.

Table 3 Nutrition and Dietary Related Characteristics of Women Using Family Planning Methods at Public Health Facilities in Ambo Town, Central Ethiopia, 2019 (n=443)

Variable	Category	Frequency	Percentage
Intake of food made from cereals/grains in the last seven days	Once	8	1.8
	Twice	31	7.0
	Often	163	36.8
	Always	241	54.4
Intake of food made from pulses in the last seven days	Never	5	1.1
	Once	39	8.8
	Twice	160	36.1
	Often	185	41.8
	Always	54	12.2
Intake of vegetables in the last seven days	Never	17	3.8
	Once	116	26.2
	Twice	168	37.9
	Often	115	26.0
	Always	27	6.1

(Continued)

Table 3 (Continued).

Variable	Category	Frequency	Percentage
Intake of fruits in the last seven days	Never	100	22.6
	Once	154	34.8
	Twice	94	21.2
	Often	73	16.5
	Always	22	5.0
Intake of meats in the last seven days	Never	116	26.2
	Once	156	35.2
	Twice	107	24.2
	Often	48	10.8
	Always	16	3.6
Intake of eggs in the last seven days	Never	83	18.7
	Once	123	27.8
	Twice	113	25.5
	Often	99	22.3
	Always	25	5.6
Intake of dairy products in the last seven days	Never	122	27.5
	Once	79	17.8
	Twice	84	19.0
	Often	79	17.8
	Always	79	17.8
Intake of sugar or honey in the last seven days	Never	58	13.1
	Once	75	16.9
	Twice	111	25.1
	Often	67	15.1
	Always	132	29.8
Intake of oil, fats or butter in the last seven days	Never	33	7.4
	Once	42	9.5
	Twice	79	17.8
	Often	78	17.6
	Always	211	47.6
Dietary diversity status	Adequate	417	94.1
	Low	26	5.9

(Continued)

Table 3 (Continued).

Variable	Category	Frequency	Percentage
Food security status	Food secure	389	87.8
	Food insecure	54	12.2
BMI(Kg/m ²)	<18.5	32	7.2
	18.5–25	411	92.8

Abbreviation: BMI, Body mass index.

Table 4 Health and Lifestyle-Related Characteristics of Women Receiving Family Planning Service in Public Health Facilities in Ambo Town, Central Ethiopia, 2019 (n=443)

Variables	Categories	Frequency	Percentage
Intake of coffee/tea after meal within 30 minutes regularly	Yes	15	3.4
	No	428	96.6
Intake of alcohol in the last seven days	Yes	44	9.9
	No	399	90.1
Smoking	Yes	0	0
	No	443	100
Chronic illness	Yes	36	8.1
	No	407	91.9
Acute illness	Yes	28	6.3
	No	415	93.7
Intestinal parasite	Yes	66	14.9
	No	377	85.1
Type of intestinal parasite	H.worm	16	23.9
	A.lumbricoids	15	22.4
	E.histolytica	11	16.4
	G.lambia	19	28.4
	E.vermicularis	2	3.0
	T.trichuria	2	3.0
	T.saginata	2	3.0

Magnitude of Anemia

In this study, the prevalence of anemia was 28.0% (95% CI: 23.9, 32.3). This prevalence was 2.84 times higher among women aged 25–35 years (AOR: 2.84, 95% CI: 1.74, 4.64) than among women aged 15–24 years (AOR: 2.84, 95% CI: 1.74, 4.64) (Figure 1).

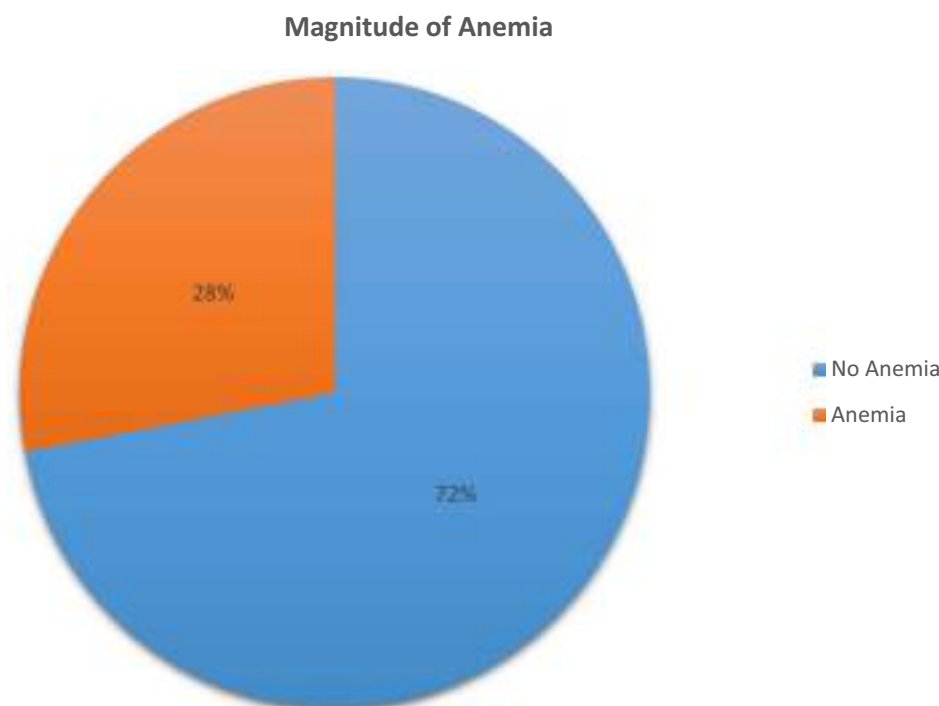


Figure 1 Magnitude of anemia among women using family planning in Ambo town, Ethiopia, 2019.

Independent Factors Associated with Anemia

In the multivariate analysis, women who used an implantable form of family planning device were 66% less chance of developing anemia [AOR= 0.34, 95% CI: (0.12, 0.96)] than women who used IUCD. Women who had not previously used any type of family planning had 2.62 (AOR=2.62, 95% CI: 1.62, 4.24) higher odds of developing anemia than women who had previously used family planning. Women from food-insecure households had twice [AOR= 2.04, 95% CI: 1.06, 3.93] higher odds of developing anemia than those from food-secure households. Regularly taking coffee or tea within 30 minutes of post-meal was associated with four [AOR= 3.85, 95% CI: 1.24, 11.92] times higher chance of developing anemia. The study also revealed that having an intestinal parasite infection substantially doubles the risk of anemia [AOR= 2.01, 95% CI: 1.12, 3.63] (Table 5).

Table 5 Factors Associated with Anemia Among Women Receiving Family Planning Service in Public Health Facilities in Ambo Town, Central Ethiopia, 2019 (n=443)

Variables	Categories	Anemia		COR 95% CI	AOR 95% CI
		Yes	No		
Age of the women	15–24	37 (29.8%)	162 (50.8%)	1	
	25–35	76 (61.3%)	140 (43.9%)	2.38 (1.51, 3.74)	2.84 (1.74, 4.64)*
	36–49	11 (8.9%)	17 (5.3%)	2.83 (1.23, 6.55)	2.48 (0.96, 6.38)
Educational status of the women	No formal education	46 (37.1%)	77 (24.1%)	2.46 (1.46, 4.15)	1.65 (0.89, 3.06)
	Primary education	44 (35.5%)	102 (32.0%)	1.78 (1.06, 2.97)	1.69 (0.97, 2.93)
	Secondary and above	34 (27.4%)	140 (43.9%)	1	

(Continued)

Table 5 (Continued).

Variables	Categories	Anemia		COR 95% CI	AOR 95% CI
		Yes	No		
Under two children in the house hold	Yes	66 (53.2%)	204 (63.9%)	1.56 (1.02, 2.37)	1.34 (0.80, 2.20)
	No	58 (46.8%)	115 (36.1%)	1	
Toilet facility	Yes	111 (89.5%)	302 (94.7%)	1	
	No	13 (10.5%)	17 (5.3%)	2.08 (0.98, 4.42)	1.25 (0.40, 3.17)
Sanitary pads used daily during menses	1–2	80 (64.5%)	235 (73.7%)	1	
	>2	44 (35.5%)	84 (26.3%)	1.54 (0.99, 2.40)	1.46 (0.90, 2.36)
Parity	None	11 (8.9%)	45 (14.1%)	1	
	1–2	66 (53.2%)	183 (57.4%)	1.48 (0.72, 3.02)	2.33 (0.91, 5.95)
	3–5	41 (33.1%)	79 (24.8%)	2.12 (0.99, 4.54)	2.17 (0.74, 6.42)
	>5	6 (4.8%)	12 (3.8%)	2.05 (0.63, 6.67)	1.17 (0.25, 5.42)
Type of family planning being used	OCP	17 (13.7%)	49 (15.4%)	0.32 (0.12, 0.85)	0.72 (0.23, 2.24)
	Injectable	75 (60.5%)	174 (54.5%)	0.40 (0.17, 0.94)	0.66 (0.25, 1.75)
	Implantable	20 (16.1%)	85 (26.6%)	0.22 (0.08, 0.56)	0.34 (0.12, 0.96)*
	IUCD	12 (9.7%)	11 (3.4%)	1	
Any other type of FP used previously	Yes	69 (55.6%)	227 (71.2%)	1	
	No	55 (44.4%)	92 (28.8%)	1.97 (1.28, 3.02)	2.62 (1.62, 4.24)*
Food security	Food secure	103 (83.1%)	286 (89.7%)	1	
	Food insecure	21 (16.9%)	33 (10.3%)	1.77 (0.98, 3.20)	2.04 (1.06, 3.93)*
Intake of coffee/tea after a meal	Yes	9 (7.3%)	6 (1.9%)	4.08 (1.42, 11.72)	3.85 (1.24, 11.92)*
	No	115 (92.7%)	313 (98.1%)	1	
Chronic illness	Yes	15 (12.1%)	21 (6.6%)	1.95 (0.97, 3.93)	1.39 (0.65, 2.98)
	No	109 (87.9%)	298 (93.4%)	1	
Intestinal parasite	Yes	28 (22.6%)	38 (11.9%)	2.16 (1.26, 3.70)	2.01 (1.12, 3.63)*
	No	96 (77.4%)	281 (88.1%)	1	

Note: *Significant at P-value <0.05.

Abbreviations: COR, Crude odds ratio; AOR, Adjusted odds ratio; CI, Confidence interval; FP, Family planning; OCP, Oral Contraceptive Pill; IUCD, Intra Uterine Device.

Discussion

Anemia affects 28% of the people in the study area. According to the study, the age of the women, history of previous family planning use, type of family planning method being used, household food security status, intestinal parasite infection, and coffee or tea consumption within thirty minutes post meal were identified as independent factors associated with anemia. Participants who were found to be anemic and harboring intestinal parasites were treated with the consultation of physicians as needed.

Anemia affects 28% of the people in this study. This finding is comparable to those in Serbia (27.7%),⁴⁶ Ethiopia in which 30.4% WRA²⁵ and 26.3% NPW³⁰ of reproductive age women were anemic. The prevalence observed in this study is higher than that reported in Brazil⁴¹ (18.6%) and Jordan (19.3%).⁴⁷ This might be due to the difference in the socio-economic status of

the population and the nutrition intervention programs the countries were undertaking. Other studies in Ethiopia reported a prevalence lower than this study.^{22,48} The difference might be attributed to the method used to assess the concentration of hemoglobin.⁴⁹ However this finding is lower than study conducted in Tanzania,⁵⁰ Sierra Leone⁵¹ and Côte d'Ivoire,⁵² and contraceptive users from Sub-Saharan African countries and Egypt,^{20,53,54} India,⁵⁵ Pakistan,² and Bangladesh.⁵⁶ The discrepancy might be because the country's inflammation exposure status in the first two⁵¹ while it might be the time gap in the latter studies. Further, the population difference in socio-economic and demographic background and the countries' higher prevalence rate might be possible reasons for the variations.

In current study, women between the ages of 25 and 35 were more affected by anemia than women between the ages of 15 and 24. This could be because women between the ages of 25 and 35 might experience repeated pregnancies and deliveries and less likely to use health care services than those aged 15–24 years, which increases the risk of anemia. The Tanzanian study and other Ethiopian investigations support this finding.^{22,50} In contrast, a Brazilian study found that being under the age of 19 increased the risk of anemia.⁴¹ This difference could be attributable to the fact that the more preventive measures among WRA has been done by Brazilian government than our country.⁴¹

The study also revealed that no previous use of family planning is connected with higher anemia. Women who had no previous use of family planning were experienced higher proportion of anemia. This is supported by Indian study.²⁸ This could be because women who previously employed family planning methods had extended birth spacing that can help them to have good adequate nutrition by inhibiting pregnancy, delivery, and breastfeeding associated risks.

The type of family planning method used has showed association with anemia in this study. Women who used an implantable type of family planning method were less affected by anemia than those who used an IUCD. This is consistent with studies in Tanzania⁵⁷ and Sub-Saharan African countries.⁵⁸ This could be because hormonal contraceptives lessen the risk of anemia by reducing excessive bleeding, which is a non-contraceptive advantage of hormonal contraceptives.^{53,57} On the other hand, an increase in menstrual flow with the use of IUCD can cause a drop in hemoglobin concentration and contribute to anemia.

Moreover, anemia was connected with household food insecurity among family planning users in this study. Similar finding was evidenced in studies conducted in Pakistan² and Bangladesh.⁵⁶ This could be because women from food-insecure households were unable to meet their higher body demands. It could be related to unbalanced and biased household food allocation that favors older boys, males, and seniors, making WRA vulnerable to anemia because of low access or insufficient intake.⁵

Further, consistent with other studies,^{22,24,25,28} the current study discovered that study participants infected with intestinal parasites had a higher proportion of anemia. This could be because intestinal parasites compete for nutrients and contribute to increased loss and malabsorption of iron, and red cell destructions.^{22,28}

Finally, the proportion of anemia was connected with drinking coffee or tea within thirty minutes after a meal. Women who reported regular consumption of either coffee or tea within 30 minutes post meal had about four times higher proportion of anemia than those who did not. This might be due to the fact that coffee and tea have inhibitory effects on iron absorption. Anemia is a moderate public health concern for women receiving family planning services based on the WHO cut-off values for NPW of reproductive age.⁴² Even though family planning services create a window of opportunities that help avoid anemia among NPW, there are missed opportunities to prevent and control anemia among family planning users.

Strengths and Limitations

The study used primary data and large sample size, and discovered a window of opportunity to prevent and control anemia among WRA. Despite these merits, women who used family planning at health posts, on the other hand, were not included in the study, which might have underestimated the prevalence of anemia. Although efforts made to reduce recall bias by probing, there may still be recall bias because study participants were asked about past occurrences. Furthermore, the time of data collection, which occurred during post-harvest in the local area, could have an impact on household food security status.

Conclusions

Anemia is a moderate public health concern among WRA receiving FP services in the study setting. Being in the age of 25–35 years, having no previous use of the FP method, household food insecurity, harboring intestinal parasites, having a regular intake of coffee or tea within 30 minutes after a meal, and using an implantable type of FP method were independent factors significantly connected with the magnitude of anemia. The study nude that opportunities to address the burden of anemia during FP services were missed. As a result, this study urges all stakeholders to prioritize incorporating and integrating proactive strategies such as nutritional screening, nutritional education, and counseling programs as standard practice in all healthcare facilities. A comparative study is also recommended to determine the magnitude of anemia from other direction in the study setting.

Abbreviations

AOR, Adjusted Odds Ratio; CI, Confidence Interval; COR, Crude Odds Ratio; EDHS, Ethiopian Demographic Health Survey; FANTA, Food and Nutrition Technical Assistance Project; FAO, Food and Agriculture Organization of the United Nations; FCS, Food Consumption Score; FP, Family planning; HDDS, Household Dietary Diversity Score; HFIAS, Household Food Insecurity Access Scale; IUCD, Intra Uterine Contraceptive Device; LMICs, Low and middle-income countries; MDD, Minimum Dietary Diversity; MDD-W, Minimum Dietary Diversity for Women of Reproductive Age; MOH, Ministry of Health; NPW, Non-pregnant women; OCP, Oral Contraceptive Pills; WDDS, Women Dietary diversity score; WRA, Women of Reproductive Age.

Data Sharing Statement

All datasets pertinent to the work are included within the article. Detailed raw data analyzed for the work reported can be available from the corresponding author upon reasonable request.

Ethical Approval and Consent to Participate

This research was carried out in accordance with the Helsinki Declaration. Haramaya University's Institutional Health Research and Ethics Review Committee granted ethical approval. All study participants aged 18–49 years provided written, voluntarily signed consent and parental informed consent was obtained for study participants aged 15–18-year-old after being informed about the study's goal, risks, and benefits. No personal identifiers used throughout the execution of the study. Standard safety measures were implemented strictly during the data collection process.

Acknowledgments

The authors expressed their heartfelt gratitude and humble appreciation to Haramaya University for giving ethical permission and financially supporting data collection. All study respondents, data collectors, and supervisors who made this study feasible were also grateful. Finally, all cooperative staffs of the Ambo town health bureau and public health facilities, as well as all individuals who contributed directly or indirectly to the accomplishment of the study were thankful.

Author Contributions

All authors made substantial intellectual contributions to the work reported, whether in the conception, study design, execution and acquisition of data, analysis, and interpretation, or in all of these parts; participated in drafting the manuscript, critical review or revision of the article; have read and approved the final version; selected and settled the journal to which the article submitted; and approved accountability in all aspects of the work reported.

Funding

This work was financially supported by Haramaya University. The funder had no role in the study selection, data collection, analysis, conclusion, interpretation and manuscript writing.

Disclosure

The authors declare that there is no financial or other competing interest that can be constructed as a potential conflict of interest in the work reported.

References

- World Health Organization. *Monitoring the Building Blocks of Health Systems, a Handbook of Indicators and Their Measurement Strategies*. World Health Organization; 2010.
- Soofi S, Khan GN, Sadiq K, et al. Prevalence and possible factors associated with anaemia, and vitamin B 12 and folate deficiencies in women of reproductive age in Pakistan, analysis of national-level secondary survey data. *BMJ open*. 2017;7(12):e018007. doi:10.1136/bmjopen-2017-018007
- UNICEF. Micronutrients; 2018. Available from: https://www.unicef.org/nutrition/index_iodine.html. Accessed November 25, 2018.
- World Health Organization, UNICEF. *Preventing and Controlling Micronutrient Deficiencies in Populations Affected by an Emergency*. Organization WH, UNICEF; 2007:2.
- Girard AW, Self JL, McAuliffe C, Olude O. The effects of household food production strategies on the health and nutrition outcomes of women and young children, a systematic review. *Paediatr Perinat Epidemiol*. 2012;26:205–222. doi:10.1111/j.1365-3016.2012.01282.x
- Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014;123(5):615–624.
- Guilbert J. The world health report 2002—reducing risks, promoting healthy life. *Educ Health*. 2003;16(2):230. doi:10.1080/1357628031000116808
- Stevens GA, Finucane MM, De-Regil LM, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011, a systematic analysis of population-representative data. *Lancet Glob Health*. 2013;1(1):e16–e25. doi:10.1016/S2214-109X(13)70001-9
- Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet*. 2011;378(9809):2123–2135. doi:10.1016/S0140-6736(10)62304-5
- World Health Organization. *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. World Health Organization; 2011.
- Kinyoki D, Osgood-Zimmerman AE, Bhattacharjee NV, Kassebaum NJ, Hay SI, Hay SI. Anemia prevalence in women of reproductive age in low-and middle-income countries between 2000 and 2018. *Nat Med*. 2021;27(10):1761–1782. doi:10.1038/s41591-021-01498-0
- WorldBank. Prevalence of anemia among women of reproductive age (% of women ages 15–49); 2018. Available from: <https://data.worldbank.org/indicator/SH.ANM.NPRG.ZS>. Accessed November 25, 2018.
- EDHS. Ethiopian demographic and health survey; 2016. Available from: <https://dhsprogram.com/pubs/pdf/FR328/FR328.pdf>. Accessed November 17, 2018.
- World Health Organization. *The Global Prevalence of Anaemia in 2011*. World Health Organization; 2015.
- World Health Organization. *Global Nutrition Policy Review, What Does It Take to Scale Up Nutrition Action?* World Health Organization; 2013.
- World Health Organization. WHO, 2014, Global nutrition targets 2025, anaemia policy brief; 2014. Available from: https://www.who.int/nutrition/topics/globaltargets_anaemia_policybrief.pdf. Accessed November 25, 2018.
- Peña-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev*. 2015;2015:7.
- Walters D, Kakietek J, Julia DE, Shekar M. An investment framework for meeting the global nutrition target for anemia; 2017.
- Habyarimana F, Zewotir T, Ramroop S. Spatial distribution and analysis of risk factors associated with anemia among women of reproductive age, case of 2014 Rwanda demographic and health survey data. *Open Public Health J*. 2018;11:1. doi:10.2174/1874944501811010425
- Hassan EO, El-Hussinie M, El-Nahal N. The prevalence of anemia among clients of family planning clinics in Egypt. *Contraception*. 1999;60(2):93–99. doi:10.1016/S0010-7824(99)00066-9
- Lakew Y, Biadgilign S, Haile D. Anaemia prevalence and associated factors among lactating mothers in Ethiopia, evidence from the 2005 and 2011 demographic and health surveys. *BMJ open*. 2015;5(4):e006001. doi:10.1136/bmjopen-2014-006001
- Asres Y, Yemane T, Gedefaw L. Determinant factors of anemia among nonpregnant women of childbearing age in southwest Ethiopia, a community based study. *Int Sch Res Notices*. 2014;2014:1–8. doi:10.1155/2014/391580
- Ejigu BA, Wencheke E, Berhane K. Spatial pattern and determinants of anaemia in Ethiopia. *PLoS One*. 2018;13(5):e0197171. doi:10.1371/journal.pone.0197171
- Gebremedhin S, Enquselassie F. Correlates of anemia among women of reproductive age in Ethiopia, Evidence from Ethiopian DHS 2005. *Ethiop J Health Dev*. 2011;25(1):22–30. doi:10.4314/ejhd.v25i1.69842
- Haidar J. Prevalence of anaemia, deficiencies of iron and folic acid and their determinants in Ethiopian women. *J Health Popul Nutr*. 2010;28(4):359–368. doi:10.3329/jhpn.v28i4.6042
- Wondt T, Bijlsma M. “The hidden hunger”, Understanding the burden of anaemia and its determinants among pregnant and non-pregnant women in Ethiopia. *African J Food Agric Nutr Dev*. 2012;12:7. doi:10.18697/ajfand.55.11015
- Derara Furgasa TD. Household food security situation in central Oromia, Ethiopia, a case study from becho wereda in southwest Shewa zone. *Glob J Hum Soc Sci*. 2016;16:2.
- Ganapathi KC, Kumar KS. A cross-sectional study of anemia among women of reproductive age group (15–49 years) in a rural population of Tamil Nadu. *Int J Med Sci Public Health*. 2017;6(3):524–530.
- Jamil S, Khan RA, Dilshad H, Fatima S. haematologic variations associated with the long term use of contraceptives in young females. *Int J Med Res*. 2014;2(5):580–586.
- Tsehayu A, Tamiru D, Mebratu W. Anaemia and associated factors among non-pregnant women of reproductive age in Ethiopia. *Afr J Midwifery Womens Health*. 2022;16(1):1–10. doi:10.12968/ajmw.2020.0045
- Debelo O, Shiferaw Y. Correlates of anemia status among women of reproductive age in Ethiopia; 2019.
- Diress G, Endalifer ML. Effect of alcohol consumption on haemoglobin level among non-pregnant reproductive age women in Ethiopia, a cross-sectional secondary data analysis of the 2016 Ethiopian demographic health survey. *BMJ open*. 2022;12(2):e046458. doi:10.1136/bmjopen-2020-046458

33. Demissie DB, Bulto GA, Terfassa TG. Involvement of male in antenatal care, birth preparedness and complication readiness and associated factors in Ambo town, Ethiopia. *J Health Med Nurs*. 2016;27(5):14–23.
34. Bulto GA, Fekene DB, Moti BE, Demissie GA, Daka KB. Knowledge of neonatal danger signs, care seeking practice and associated factors among postpartum mothers at public health facilities in Ambo town, Central Ethiopia. *BMC Res Notes*. 2019;12(1):549. doi:10.1186/s13104-019-4583-7
35. FAO aF. *Minimum Dietary Diversity for Women, a Guide for Measurement*. Rome: University of California; 2016.
36. Mohammadi F, Omidvar N, Houshiar-Rad A, Khoshfetrat MR, Abdollahi M, Mehrabi Y. Validity of an adapted household food insecurity access scale in urban households in Iran. *Public Health Nutr*. 2012;15(1):149–157. doi:10.1017/S1368980011001376
37. FAO F. *Minimum Dietary Diversity for Women, a Guide for Measurement*. Vol. 82. Rome: FAO; 2016.
38. Swindale A, Bilinsky P. Development of a universally applicable household food insecurity measurement tool, process, current status, and outstanding issues. *J Nutr*. 2006;136(5):1449s–1452s. doi:10.1093/jn/136.5.1449S
39. Coates J, Swindale A, Bilinsky P. Household Food Insecurity Access Scale (HFIAS) for measurement of food access, indicator guide, version 3; 2007.
40. INDDEx Project. *Data4Diets, Building Blocks for Diet-Related Food Security Analysis*. Boston, MA: Tufts University; 2018.
41. Bezerra A, Leal V, Lira P, et al. Anemia and associated factors in women at reproductive age in a Brazilian Northeastern municipality. *Rev Bras Epidemiol*. 2018;21:e180001–e180001. doi:10.1590/1980-549720180001
42. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity, 2011 and for adjustment; 2011. Available from: <https://www.who.int/vmnis/indicators/haemoglobin.pdf>. Accessed November 25, 2018.
43. Gonete KA, Tariku A, Wami SD, Derso T. Prevalence and associated factors of anemia among adolescent girls attending high schools in Dembia District, Northwest Ethiopia, 2017. *Arch Public Health*. 2018;76(1):79. doi:10.1186/s13690-018-0324-y
44. World Health Organization. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Document Reference WHO. NMH/NHD/MNM/11.1*. Geneva S; 2011. Available from: <http://www.who.int/entity/vmnis/indicators/haemoglobin>. Accessed February 2, 2023.
45. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and mineral nutrition information system, 1993–2005. *Public Health Nutr*. 2009;12(4):444–454. doi:10.1017/S1368980008002401
46. Rakic L, Djokic D, Drakulovic M, Pejic A, Radojicic Z, Marinkovic M. Risk factors associated with anemia among Serbian non-pregnant women 20 to 49 years old. A cross-sectional study. *Hippokratia*. 2013;17(1):47.
47. Abdo N, Douglas S, Batieha A, et al. The prevalence and determinants of anaemia in Jordan. *East Mediterr Health J*. 2018;25(5):341–349. doi:10.26719/emhj.18.047
48. Kibret KT, Chojenta C, D'Arcy E, Loxton D. Spatial distribution and determinant factors of anaemia among women of reproductive age in Ethiopia, a multilevel and spatial analysis. *BMJ open*. 2019;9(4):e027276. doi:10.1136/bmjopen-2018-027276
49. Adam I, Ahmed S, Mahmoud MH, Yassin MI. Comparison of HemoCue[®] hemoglobin-meter and automated hematology analyzer in measurement of hemoglobin levels in pregnant women at Khartoum hospital, Sudan. *Diagn Pathol*. 2012;7(1):30. doi:10.1186/1746-1596-7-30
50. Msemo OA, Bygbjerg IC, Møller SL, et al. Prevalence and risk factors of preconception anemia, A community based cross sectional study of rural women of reproductive age in northeastern Tanzania. *PLoS One*. 2018;13(12):e0208413. doi:10.1371/journal.pone.0208413
51. Wirth JP, Rohner F, Woodruff BA, et al. Anemia, micronutrient deficiencies, and malaria in children and women in Sierra Leone prior to the Ebola outbreak-findings of a cross-sectional study. *PLoS One*. 2016;11(5):e0155031. doi:10.1371/journal.pone.0155031
52. Rohner F, Northrop-Clewes C, Tschannen AB, et al. Prevalence and public health relevance of micronutrient deficiencies and undernutrition in pre-school children and women of reproductive age in Cote d'Ivoire, West Africa. *Public Health Nutr*. 2014;17(9):2016–2028. doi:10.1017/S136898001300222X
53. Gebremedhin S, Asefa A. Association between type of contraceptive use and haemoglobin status among women of reproductive age in 24 sub-Saharan Africa countries. *BMJ Sex Reprod Health*. 2018;bmjsrh-2018–200178. doi:10.1136/bmjsrh-2018-200178
54. Haile ZT, Kingori C, Teweldeberhan AK, Chavan B. The relationship between history of hormonal contraceptive use and iron status among women in Tanzania, A population-based study. *Sex Reprod Healthc*. 2017;13:97–102. doi:10.1016/j.srhc.2017.07.003
55. Naaz S, Khan AA, Begum W, Mukhtar F, Ainuddin N, Tarannum S. Prevalence of anemia in reproductive age females in Aligarh, a retrospective study. *Group*. 2005;18:42.
56. Kamruzzaman M, Rabbani M, Saw A, Sayem M, Hossain M. Differentials in the prevalence of anemia among non-pregnant, ever-married women in Bangladesh, multilevel logistic regression analysis of data from the 2011 Bangladesh Demographic and Health Survey. *BMC Women's Health*. 2015;15(1):1–8. doi:10.1186/s12905-015-0211-4
57. Wilunda C, Massawe S, Jackson C. Determinants of moderate-to-severe anaemia among women of reproductive age in Tanzania, analysis of data from the 2010 Tanzania demographic and health survey. *Trop Med Int Health*. 2013;18(12):1488–1497. doi:10.1111/tmi.12199
58. Gebremedhin S, Asefa A. Association between type of contraceptive use and haemoglobin status among women of reproductive age in 24 sub-Saharan Africa countries. *BMJ Sex Reprod Health*. 2019;45(1):54–60.



MicroRNAs as Quality Assessment Tool in Stored Packed Red Blood Cell in Blood Banks

Zewudu Mulatie, Melak Aynalem & Solomon Getawa

To cite this article: Zewudu Mulatie, Melak Aynalem & Solomon Getawa (2023) MicroRNAs as Quality Assessment Tool in Stored Packed Red Blood Cell in Blood Banks, Journal of Blood Medicine, , 99-106, DOI: [10.2147/JBM.S397139](https://doi.org/10.2147/JBM.S397139)

To link to this article: <https://doi.org/10.2147/JBM.S397139>



© 2023 Mulatie et al.



Published online: 08 Feb 2023.



Submit your article to this journal



Article views: 277



View related articles



View Crossmark data



Citing articles: 1 View citing articles

MicroRNAs as Quality Assessment Tool in Stored Packed Red Blood Cell in Blood Banks

Zewudu Mulatie¹, Melak Aynalem², Solomon Getawa²

¹Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, Desie, Ethiopia; ²Department of Hematology and Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Correspondence: Zewudu Mulatie, Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, P.O. box: 1145, Desie, Ethiopia, Tel +251945274251, Email zewudumulatie@gmail.com

Abstract: Micro-ribonucleic acids are control gene expression in cells. They represent the changed cellular states that occur can be employed as biomarkers. Red blood cells alter biochemically and morphologically while they are being stored, which could be detrimental to transfusion. The effect of storage on the erythrocyte transcriptome is not mostly investigated. Because adult erythrocytes lack a nucleus, it has long been assumed that they lack deoxyribonucleic acid and ribonucleic acid. On the other hand, erythrocytes contain a diverse range of ribonucleic acids, of which micro-ribonucleic acids are key component. Changes in this micro-ribonucleic acid protect cells from death and adenine triphosphate depletion, and they are linked to specific storage lesions. As a result, changes in micro-ribonucleic acid in stored erythrocytes may be used as a marker to assess the quality and safety of stored erythrocytes. Therefore, this review aims to review the role of microRNA in stored packed red blood cells as quality indicator. Google Scholar, PubMed, Scopus, and Z-libraries are used for searching articles and books. The article included in this paper was written in the English language and had the full article. During long storage of RBCs, miR-16-2-3p, miR-1260a, miR-1260b, miR-4443, miR-4695-3p, miR-5100, let-7b, miR-16, miRNA-1246, miR-31-5p, miR-203a, miR-654-3p, miR-769-3p, miR-4454, miR-451a and miR-125b- 5p are up regulated. However, miR-96, miR-150, miR-196a, miR-197, miR-381 and miR-1245a are down regulated after long storage of RBCs. The changes of this microRNAs are linked to red blood cell lesions. Therefore, micro-ribonucleic acids are the potential quality indicator in stored packed red blood cells in the blood bank. Particularly, micro-ribonucleic acid-96 is the most suitable biomarker for monitoring red blood cell quality in stored packed red blood units.

Keywords: blood bank, microRNA, packed red blood cell

Introduction

Blood transfusions save hundreds of lives around the world per day. The most commonly transfused blood component worldwide is concentrated red blood cells (RBC).¹ Blood transfusions are one of the most common clinical therapeutic procedures for acute or chronic blood loss, with approximately 85 million RBC units transfused each year around the world.² Blood and blood component storage allows for better control of blood supply safety, adequacy, and management.³ Red blood cells can now be kept in a solution of saline adenine-glucose-mannitol (SAGM) as a combination anticoagulant and energy source for up to 6 weeks at a temperature of +4°C for an adequate and available blood supply, however storage may compromise the efficacy and quality of blood components.⁴

During storage, blood cells continue their metabolic activity, releasing byproducts into the medium and undergoing biologic and immunologic alterations that may have an impact on RBC function and survival.^{5,6} Despite recent improvements in blood storage conditions, a wide range of detrimental molecular, biochemical, and physical modifications known as “storage lesions” have been observed during preservation.^{1,5} Metabolic effects, shape changes, membrane loss, rheological alterations, losses of membrane carbohydrates, oxidative damage to lipids and proteins, changes in oxygen affinity, and changes in oxygen delivery are all part of the storage lesion that red blood cells go through.

Table 1 Summary of the Expression of miRNA in Stored Packed RBCs

List of MiRNA	Expression	Function	Reference
MicroRNA-16	Up	Promote cell apoptosis	[56,58]
MiRNA-4443	Up	Promote apoptosis	[56]
MiRNA-4695-3p	Up	Inhibition of apoptosis	[56]
MiRNA-5100	Up	Enhance the apoptosis	[56]
Let-7b	Up	Integrity of the RBC membrane	[58]
MiRNA-1246	Up	Inhibiting survival and promoting apoptosis	[57]
MiRNA-31-5p	Up	Promote apoptosis	[58]
MiRNA-203a	Up	Promote of cell apoptosis	[58]
MiRNA-654-3p	Up	Promote apoptosis	[58]
MiRNA-769-3p	Up	Enhanced apoptosis	[58]
MiRNA-4454	Up	Changes in micro-environment of stored RBC	[59]
MiRNA-451a	Up	Protecting RBCs from oxidative stress and homeostasis of RBCs	[59]
MiRNA-125b-5p	Up	Inhibition apoptosis	[59]
MiRNA-96	Down	Inhibition of apoptosis	[58,60]
MiRNA-150	Down	Inhibition of apoptosis	[58]
MiRNA-196a	Down	Inhibition of apoptosis	[58]
MiRNA-197	Down	Inhibition of apoptosis	[58]
MiRNA-381	Down	Promoting apoptosis	[82]
MiRNA-1245a	Down	Promoting apoptosis	[82]

Additionally, there are concerns associated with shorter RBC lifespan, increased RBC adherence to endothelium, potassium buildup, and the loss of active proteins, lipids, and microvesicles.⁷

Storage damages include changes in cell flexibility and a decrease in glucose, diphosphoglycerate, and adenine triphosphate (ATP) levels, as well as acidosis caused by lactic acid accumulation. Deactivation of the sodium/potassium ATPase pump results in a drop in sodium and a rise in potassium levels in plasma.⁴ Other morphological and molecular alterations, as well as oxidative damage to lipids, proteins, and carbohydrates, contributed to the spherocytic shape. Spherical cells have a lower surface area-to-volume ratio than discoid cells, which leads to higher osmotic fragility and decreased cell deformability and survival.⁸

The two key elements that influence the process of causing a red blood cell storage lesion are the length of storage period and donor variability. Even when the same amount of time and ex vivo storage conditions are used, donor variation may affect the quality of the red blood cell units obtained from various blood donors. Donor genetic and environmental characteristics are directly related to donor variation.^{9,10} Red blood cell storage capacity may be impacted by the inherent traits of blood donors (RBCs). Hemolysis, redox, and metabolic characteristics of stored RBCs as well as their post-transfusion recovery have been demonstrated to be influenced by environmental (alcohol use,¹¹ smoking¹² and genetic (sex,¹³ ethnicity,¹⁴ hemoglobin (Hb) mutation.¹⁵ The increase in red blood storage lesion was caused by factors including blood donors' aging, smoking, BMI, and decreased level of physical activity.¹⁶ Additionally, having a male sex, being Asian or African American, having sickle cell disease, thalassemia, or lacking glucose-6-phosphate

dehydrogenase may alter RBC rheology or antioxidant capability and compromise RBC recovery during storage and after transfusion.^{15,17,18}

RBCs' homeostasis is affected by storage on three levels: energy metabolism, redox metabolism, and cell membrane, leading to a clearly characterized phenotype of morphologic, structural, and functional alterations. This phenotype is characterized by loss of normal shape due to the buildup of free hemoglobin, heme, lactate, oxidized glutathione, malondialdehyde, potassium, inflammatory lipids, and extracellular vesicles, including microvesicles. Additionally, preserved packed red blood cells showed decreased PH, glutathione, phospholipids, cholesterol, and 2,3-diphosphoglycerate levels.^{7,8} Additionally, improper ATP-centered metabolism and oxidative stress contribute to RBC storage lesions (RBCSL). High energy phosphate compound metabolites are used as energy sources to preserve membrane integrity and, hence, RBC survival. This process causes the RBCs to lose energy and valuable deformability, physiological surface area, topography, and shape.¹⁹

In general, these alterations have an impact on the quality of RBCs that have been preserved for a long time. Even when transfused within the 42 days, stored RBC has a worse quality including the higher fraction of RBC eliminated after transfusion, reduced oxygen delivery capability, reduced deformability and adverse clinical consequences.^{20–25} Also, these changes may have been linked to a negative impact on transfusion.^{8,26} Transfusions of old RBCs have been linked to greater rates of morbidity and mortality in specific patient groups, particularly in newborns and infants after massive transfusions. Transfusions of RBC with elevated potassium levels have been linked to cardiac hyperkalemia and neonatal arrhythmia.^{4,8} Prolonged RBC preservation has been linked to an increased risk of problems and poor clinical results. Patients with heart disease who had “older” RBC transfusions may develop thrombotic problems.²⁷ Prolonged RBC preservation in trauma patients may raise the risk of deep vein thrombosis and bacterial infections.^{28,29} The alterations in RBCs and bioactive chemicals accumulated during storage are thought to be the cause of these problems.³⁰ Hemoglobin or hematocrit, percent hemolysis, PH, lactate, electrolyte panels, and 2,3-diphosphate glycerides are among the RBC quality indicators tested by conventional techniques. The novel method for checking the quality of packed RBCs in blood banks is micro-ribonucleic acid (miRNA).³¹

MicroRNAs are short, non-coding RNA molecules that control how genes are expressed in cells. They are 22 nucleotide lengths in their mature state with great stability and long half-life. The miRNA molecule binds to a messenger RNA (mRNA) with complementarity in the 3'-untranslated region, causing translation to be disrupted and gene expression to be prevented.³² In humans, there are more than 2500 mature miRNAs, resulting in a greater family of microRNAs.³³ Those miRNAs are essential for several biological processes, including cell proliferation, tissue development, cell growth, apoptosis,³⁴ signal transmission, immunomodulation, and hematopoiesis.³⁵ MiRNAs are thought to regulate more than 60% of human protein-coding genes.³⁶

For many years, microRNAs have been successfully used as diagnostic markers for a variety of diseases.³⁷ It is possible that changes in miRNA expression levels cause disease and cellular abnormalities, or that diseases cause dysregulated miRNA expression.³⁸ MicroRNAs are involved in a wide range of biological processes, and it has been suggested that when they are dysfunctional, human illnesses can result.^{36,39,40} They play a function in the inflammatory response, solid cancer and leukemia, and neurological illnesses and are involved in the pathogenesis of a variety of diseases.⁴¹ It can be used as valuable clinical biomarkers for diagnosis and prognosis of specific diseases, as well as monitoring therapy responses, due to their unique qualities of disease specificity, high stability, and accessibility.⁴²

Researchers have studied the physiological and biochemical alterations that occur in stored RBCs. RBC must use its accessible posttranscriptional machinery in the cytoplasm to be alive and functional, particularly during in vitro storage, and to control all essential physiologic functions because it is enucleated.⁴³ Despite the lack of ribosomal and large-sized RNAs in mature RBCs, miRNAs are numerous and diverse in mature erythrocytes.⁴⁴ MicroRNAs are non-coding tiny single RNA molecules that play a key role in a variety of biological processes in RBCs such as cell differentiation, proliferation, and apoptosis. As a result, miRNA alterations in stored RBCs could be used as biomarkers for storage lesions. Due to the linkage between miRNA and its target mRNAs in terms of gene regulation, the underlying processes of miRNA as a quality indicator in stored packed RBCs may offer insight into related RBCSL.⁴⁵ Researchers have so far paid little attention to the comprehensive study of RBCSL in terms of the exact nature of the occurrence and the molecular components involved.⁴⁶ Therefore, this review aims to review the role of miRNA as a quality indicator for

stored packed RBCs in blood banks. For preparing this review, Google Scholar, PubMed, Scopus and Z-libraries were used for searching articles and books. The keywords such as, Blood bank, microRNA and red blood cells. The article included in this paper was written in the English language and had the full article. Articles that are outdated are excluded from the review. This means the article included only published over 2000 G.C. The reference was entered by endnote Version 20 in Vancouver style.

MicroRNA Expression in Stored Packed RBC as Quality Tool

Compared to other blood cells, mature RBCs have an abundance of miRNAs, which are the negative regulators of cellular mRNAs.⁴⁵ During storage, the mRNA and miRNA present in stored RBCs undergo morphological and molecular alterations. The miRNA and mRNA expression profiles are altered during storage in RBC. It is reasonable to postulate that miRNAs provide translational regulation of existing mRNAs in these cells. These decisional steps within the stored RBCs will ultimately determine the fate of the RBCs, that is survival during storage by resisting the onset of physiological changes or ultimately undergoing cell death.³⁸

The effect of storage on the erythrocyte transcriptome has not been thoroughly investigated. Because adult erythrocytes lack a nucleus, it has long been assumed that they lack deoxy nucleotide acid (DNA) and RNA. On the other hand, erythrocytes contain a diverse range of RNAs, of which miRNAs are a key component, and these miRNAs persist beyond terminal differentiation.^{47,48} Changes in these miRNAs protect cells from death and ATP depletion, and they are linked to specific storage lesions.⁴⁹ As a result, changes in miRNA in stored erythrocytes may be used as a marker to assess the quality and safety of stored erythrocytes.⁵⁰ MiRNAs should be considered when looking for novel and stable biomarkers in stored packed RBCs for a number of reasons, including the fact that they are very stable in RBCs, some miRNAs can only be expressed in certain cells, and miRNA levels are simple to measure using common laboratory methods, even more advanced and powerful ones.⁵¹ The following facts also pertain to miRNAs and cellular apoptosis as they relate to the quality of RBCs that have been stored. The first is that mature RBCs contain a large amount of miRNAs. White blood cell miRNAs have little effect on the second RBC miRNA study because RBC miRNAs create a significant abundance of white blood cells.⁴⁴ The third is that miRNAs are widely known for controlling how genes and mRNAs expressed within cells, particularly those involved in differentiation and apoptosis.⁴⁵

The RBC storage lesion is frequently accompanied by considerable transcriptome changes. The miRNAs must be expressed at a high level and target to have a biological effect, and they may even serve a unique role in anucleated cells' physiological activities. As a result, miRNAs are suitable prospective markers for investigating storage lesions in RBC storage because of their abundance and their stable structure.⁵² As cells differentiate into reticulocytes, the nuclei are expelled, but translation activities and cytoplasmic RNAs (including mRNAs and miRNAs) are still observable.⁵³

Understanding how miRNAs work in RBCs as they are being stored may shed light on the mechanisms underlying storage defects.⁵⁴ Through mRNA degradation or translation inhibition, microRNAs regulate cellular processes like apoptosis. This regulatory role of the miRNA is more pertinent to understanding the events connected to RBC stored damage during storage. These cells must rely on the available cytoplasmic posttranscriptional regulatory systems for survival because they are enucleated and have lost their transcriptional regulatory mechanisms.⁵⁵ As a result, these miRNAs are anticipated to play an important role in erythroid cell posttranscriptional regulation. The expression of numerous miRNAs can substantially change during storage, according to RBC profiling studies, and apoptosis-associated miRNAs have been discovered.^{38,45}

The miRNA of RBCs changes dramatically during storage. For example, the up regulation of miRNA-16-2-3p, miRNA-1260a, miRNA-1260b, miRNA-4443, miRNA-4695-3p, miRNA-5100, lethal (let)-7b, miRNA-16, miRNA-1246, miRNA-31-5p, miRNA-203a, miRNA-654-3p, miRNA-769-3p, miRNA-4454, miRNA-451a and miRNA-125b-5p occurred during long-term preservation of erythrocytes.^{45,56-59} Although miRNA-96, miRNA-150, miRNA-196a, miRNA-197, miRNA-381, and miRNA-1245a are down regulated after 20 days of storage.^{45,50,58} The significance of miRNAs in RBC storage lesions has been confirmed by microRNA dysregulation, and this finding raises the possibility that miRNAs in RBCs serve as possible quality markers in stored RBCs.⁴⁵

Each miRNA in stored RBCs has many functions. Also, the mechanism of how the miRNA is used as the quality tool for stored packed RBCs is based on their function. Based on the research using the target scan tool revealed that let-7b, miRNA-16, miRNA-96, miRNA-196a, miRNA-150, and miRNA-197 each can target on 819, 733, 787, 211, 142, and 140 genes of mRNA, respectively. At least one gene is related to the RBC membrane and apoptosis and targeted by each

miRNA.⁶⁰ MicroRNA-96 appears to target at least 17 conserved mRNAs (genes) associated with RBC membrane and apoptosis.⁴⁵ Potential targets for miR-96 included septin family genes, cathepsin B, docking protein 4, CAPNS1, caspase 2, and apoptosis-associated tyrosine kinase. The role of miRNA-96 could be the inhibition of apoptosis in mature RBCs. An increase in miRNA-96 expression level in RBCs may inhibit apoptosis. Thereby, this inhibition of apoptosis may result in the accumulation of lipid peroxidation and decrease in antioxidant resources within the RBCs, which can affect the quality of RBC that would be transfused to a blood recipient. Because the accumulation of lipid peroxidation is one key components of RBC storage lesion.⁶⁰ MicroRNA-196a was found to protect stored RBCs from cell death and ATP loss. The variations in miRNA during erythrocyte storage are linked to certain storage lesions. As a result, miRNA has the potential to be used as a biomarker to assess the quality and safety of RBCs.⁵⁵ MicroRNA-96 is most likely a good candidate for testing as a potential biomarker for evaluating the quality of RBCs in stored blood units.⁶⁰

The most prevalent miRNAs that have been found to be intracellularly linked to aragonite 2 in 24-hour-stored RBCs are miRNA-451a, miRNA-16, let-7, and miRNA-486-5p.⁶¹ In most cells and tissues, miRNA-16 is one of the most prevalently cloned miRNAs. It was discovered to be stable and abundant in RBC storage.⁶² It is a significant miRNA that participates in a variety of biological activities., includes encouraging cell apoptosis and suppresses cell growth.^{63,64} Lethal-7 was one of the miRNAs which are crucial for cell maturation and development,⁶⁵ one of the useful markers of RBC storage is lethal-7b, and the storage of RBCs for up to 40 days causes its levels to rise. It demonstrates that this miRNA is involved in preserving the RBC membrane's integrity.⁴⁵

Researchers have determined that miRNA-451a plays a crucial role in the homeostasis of RBCs. Furthermore, it has been observed that miRNA-451a positively controls terminal erythroid development, inhibits the expression of 14-3-3zeta, and protects RBCs from oxidative stress.^{66,67} The significant regulatory roles for miRNA-381 and miRNA-1245a are inducing apoptosis.^{68,69} After 1 week of storage, the expression levels of miRNA-381 and miRNA-1245a in RBC were reduced, suggesting that these two miRNAs can resist the negative effects of storage damage. MicroRNA-381 and miRNA-1245a may therefore be employed as indicators for RBC storage products.⁵⁰ According to reports, miRNA-150-3p is crucial in preventing the apoptotic process. Additionally, it has been demonstrated to play a role in differentiating erythrocytes.⁷⁰ MicroRNA-769-3p overexpression may be crucial for RBC survival and promotes apoptosis.⁷¹ The function of miRNA-1246 is to promote apoptosis while suppressing cellular survival.⁷² The function of miRNA-4695-3p is to prevent RBCs from apoptosis.⁷³ The cell growth is inhibited by miRNA-4443.⁷⁴

For the miRNA molecule with multiple functions, miRNA-125b is small and non-coding. It is one of the earliest miRNAs to be discovered. It is essential to many functions, including the control of RBCs' differentiation, and apoptosis.⁷⁵ MicroRNA-769-3p overexpression dramatically reduced cell growth and increased apoptosis.⁷⁶ The miRNA-203a-3p has many impacts, including reducing cell growth and promoting apoptosis.⁷⁷ MiRNA-654-3p overexpression could induce apoptosis by targeting the mRNA for the ras protein activator like 2 genes.⁷⁸ The overexpression of miRNA-5100 can induce apoptosis.⁷⁹ The fact that these miRNAs have a direct link to the mRNAs that are expected to be their apoptosis-related targets will also demonstrate that there was a connection relevant to the storage lesions in RBCs. Given their relationship to mRNA, it would make sense to think that these miRNAs will serve as appropriate indications for storage lesions. Therefore, this microRNA profile may indicate certain types of storage lesions in RBCs Table 1.³⁸

MiRNAs are a novel method for confirming the quality of packed RBCs in blood banks. But, they have some drawbacks. These are the lack of reproducibility across technologies for biomarker discovery in RBCs, some miRNAs are released from blood cells during the storage period and could lead to false results,⁸⁰ the extraction and analysis of miRNAs from the RBCs requires long times and needs experienced personnel, needs sophisticated laboratories and expensive for detections of miRNA in stored packed RBC.⁸¹ Finally, all miRNA findings need additional validation processes and an exact standardization of all pre-analytical and analytical procedures before being applied to clinical practice in order to account for any potential technical biases.

Conclusion

Red blood cells have a comprehensive storage lesion that involves metabolic consequences, shape alterations, membrane loss, rheological changes, losses of membrane carbohydrates, and oxidative damage to lipids. Additionally, the proteins'

modifications to oxygen affinity and delivery, the RBCs' enhanced endothelial adherence and shorter lifespan, as well as the incidental dangers of potassium buildup and the loss of active proteins, lipids, and microvesicles. The miRNA-16-2-3p, miRNA-1260a, miRNA-1260b, miRNA-4443, miRNA-4695-3p, miRNA-5100, let-7b, miRNA-16, miRNA-1246, miRNA-31-5p, miRNA-203a, miRNA-654-3p, miRNA-769-3p, miRNA-4454, miRNA-451a and miRNA-125b-5p increased during long storage of erythrocytes. However, miRNA-96, miRNA-150, miRNA-196a, miRNA-197, miRNA-381 and miRNA-1245a are down regulated after long storage of RBCs. MicroRNA alterations in stored RBCs can be used as biomarkers for storage lesions. Particularly, miRNA-96 is likely the best candidate to be tested as a potential biomarker for assessing the quality of RBCs in blood units that have been kept. Therefore, recommended for blood bank, the microRNAs are tested as a potential quality biomarker for packed red blood cell. Also, the integration of both biochemical and microRNA detection test is used as quality indicator of packed RBCs. Recommended for researchers, there needs a further study about the role of miRNA as quality indicator for stored packed RBCs and the mechanism how the miRNA used as a quality indicator for stored RBCs. There is a limited study in the role of miRNA as quality tool for stored RBCs, and the mechanism is also not well addressed.

Abbreviations

ATP, adenosine tri phosphate; DNA, deoxyribonucleic acid; Let, lethal; MiRNAs, microribonucleic acid; MRNA, messenger ribonucleic acid; NGS, next-generation sequencing; PCR, polymerase chain reaction; RBC, red blood cell; RBCSL, red blood cell storage lesion; RT, reverse transcriptase; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SAGM, saline-adenine-glucose-mannitol.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Zimrin B, Hess R. Current issues relating to the transfusion of stored red blood cells. *Int J Blood Transfus*. 2009;96(2):93–103.
2. Carson J, Guyatt G. AABB releases clinical practice guidelines on red blood cell transfusion and storage. *J Am Med Assoc*. 2021;3(1):28–32.
3. Antonelou H, Seghatchian J. Insights into red blood cell storage lesion: toward a new appreciation. *Transfus Apher Sci*. 2016;55(3):292–301. doi:10.1016/j.transci.2016.10.019
4. Hess R. Measures of stored red blood cell quality. *Int Soc Blood Transfus*. 2014;107(1):1–9.
5. Obrador R, Musulin S, Hansen B. Red blood cell storage lesion. *J Vet Emerg Crit Care*. 2015;25(2):187–199. doi:10.1111/vec.12252
6. Cummings A, Abelson L, Rozanski A, Sharp R. The effect of storage on ammonia, cytokine, and chemokine concentrations in feline whole blood. *J Vet Emerg Crit Care*. 2016;26(5):639–645. doi:10.1111/vec.12510
7. Hess R. Red cell changes during storage. *Transfus Apher Sci*. 2010;43(1):51–59. doi:10.1016/j.transci.2010.05.009
8. D'Alessandro A, Kriebardis G, Rinalducci S, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. *Transfusion*. 2015;55(1):205–219. doi:10.1111/trf.12804
9. Antonelou MH, Seghatchian J. Insights into red blood cell storage lesion: toward a new appreciation. *Transfus Apher Sci*. 2016;55(3):292–301.
10. Tzounakas VL, Kriebardis AG, Papassideri IS, Antonelou MH. Donor-variation effect on red blood cell storage lesion: a close relationship emerges. *Proteomics Clin Appl*. 2016;10(8):791–804. doi:10.1002/prca.201500128
11. D'Alessandro A, Fu X, Reisz JA, et al. Ethyl glucuronide, a marker of alcohol consumption, correlates with metabolic markers of oxidant stress but not with hemolysis in stored red blood cells from healthy blood donors. *Transfusion*. 2020;60(6):1183–1196. doi:10.1111/trf.15811
12. Stefanoni D, Fu X, Reisz JA, et al. Nicotine exposure increases markers of oxidant stress in stored red blood cells from healthy donor volunteers. *Transfusion*. 2020;60(6):1160–1174. doi:10.1111/trf.15812
13. Tzounakas VL, Anastasiadi AT, Drossos PV, et al. Sex-related aspects of the red blood cell storage lesion. *Blood Transfus*. 2021;19(3):224–236. doi:10.2450/2020.0141-20
14. D'Alessandro A, Fu X, Kanas T, et al. Donor sex, age and ethnicity impact stored red blood cell antioxidant metabolism through mechanisms in part explained by glucose 6-phosphate dehydrogenase levels and activity. *Haematologica*. 2021;106(5):1290–1302. doi:10.3324/haematol.2020.246603
15. Tzounakas VL, Anastasiadi AT, Stefanoni D, et al. Beta thalassemia minor is a beneficial determinant of red blood cell storage lesion. *Haematologica*. 2022;107(1):112–125. doi:10.3324/haematol.2020.273946
16. Kanas T, Lanteri MC, Page GP, et al. Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-Omics study. *Blood Adv*. 2017;1(15):1132–1141. doi:10.1182/bloodadvances.2017004820
17. Raciti PM, Francis RO, Spitalnik PF, Schwartz J, Jhang JS. Acquired hemoglobin variants and exposure to glucose-6-phosphate dehydrogenase deficient red blood cell units during exchange transfusion for sickle cell disease in a patient requiring antigen-matched blood. *J Clin Apher*. 2013;28(4):325–329. doi:10.1002/jca.21255
18. Francis RO, Jhang J, Hendrickson JE, Zimring JC, Hod EA, Spitalnik SL. Frequency of glucose-6-phosphate dehydrogenase-deficient red blood cell units in a metropolitan transfusion service. *Transfusion*. 2013;53(3):606–611. doi:10.1111/j.1537-2995.2012.03765.x

19. Lang E, Pozdeev I, Xu C, et al. Storage of erythrocytes induces suicidal erythrocyte death. *Cell Physiol Biochem*. 2016;39(2):668–676. doi:10.1159/000445657
20. Qu L, Triulzi J. Clinical effects of red blood cell storage. *Cancer Control*. 2015;22(1):26–37. doi:10.1177/107327481502200105
21. Koch G, Figueroa I, Li L, Sabik F, Mihaljevic T, Blackstone H. Red blood cell storage: how long is too long? *Ann Thorac Surg*. 2013;96(5):1894–1899. doi:10.1016/j.athoracsur.2013.05.116
22. Refaai A, Blumberg N. The transfusion dilemma—weighing the known and newly proposed risks of blood transfusions against the uncertain benefits. *Best Pract Res Clin Anaesthesiol*. 2013;27(1):17–35. doi:10.1016/j.bpa.2012.12.006
23. Flegel A, Natanson C, Klein G. Does prolonged storage of red blood cells cause harm? *Br J Haematol*. 2014;165(1):3–16. doi:10.1111/bjh.12747
24. Jy W, Ricci M, Shariatmadar S, Gomez-Marin O, Horstman H, Ahn S. Microparticles in stored red blood cells as potential mediators of transfusion complications. *Transfusion*. 2011;51(4):886–893. doi:10.1111/j.1537-2995.2011.03099.x
25. Hod A, Brittenham M, Billote B, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. *Blood*. 2011;118(25):6675–6682. doi:10.1182/blood-2011-08-371849
26. Doctor A, Spinella P. Effect of processing and storage on red blood cell function in vivo. *Semin Perinatol*. 2012;36(4):248–259. doi:10.1053/j.semperi.2012.04.005
27. Czubak-Prowizor K, Rywaniak J, Zbikowska M. Red blood cell supernatant increases activation and agonist-induced reactivity of blood platelets. *Thromb Res*. 2020;196(3):543–549. doi:10.1016/j.thromres.2020.10.023
28. Spinella C, Carroll L, Staff I, et al. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. *Crit Care*. 2009;13(5):R151. doi:10.1186/cc8050
29. Juffermans P, Vlaar P, Prins J, Goslings C, Binnekade M. The age of red blood cells is associated with bacterial infections in critically ill trauma patients. *Blood Transfus*. 2012;10(3):290–295. doi:10.2450/2012.0068-11
30. Sparrow L. Red blood cell storage and transfusion-related immunomodulation. *Blood Transfus*. 2010;8(3):26–30.
31. D'Alessandro A, Dzieciatkowska M, Nemkov T, Hansen KC. Red blood cell proteomics update: is there more to discover? *Blood Transfus*. 2017;15(2):182–187. doi:10.2450/2017.0293-16
32. Dave V, Ngo T, Pernestig A, et al. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. *Lab Invest*. 2019;99(4):452–469. doi:10.1038/s41374-018-0143-3
33. Kozomara A, Griffiths S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014;42(1):68–73. doi:10.1093/nar/gkt1181
34. Esquela A, Slack F. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer*. 2006;6(4):259–269. doi:10.1038/nrc1840
35. Undi B, Kandi R, Gutti K. MicroRNAs as haematopoiesis regulators. *Adv Hematol*. 2013;20(13):695–754.
36. Friedman C, Farh K, Burge B, Bartel P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19(1):92–105. doi:10.1101/gr.082701.108
37. Lu M, Zhang Q, Deng M, et al. An analysis of human microRNA and disease associations. *PLoS One*. 2008;3(10):34. doi:10.1371/journal.pone.0003420
38. Ryan P, Atreya C. Blood cell microRNAs: what are they and what future do they hold? *Transfus Med Rev*. 2011;25(3):247–251. doi:10.1016/j.tmr.2011.01.005
39. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. *Nat Rev Genet*. 2007;8(2):93–103. doi:10.1038/nrg1990
40. Kim J, Inoue K, Ishii J, et al. A microRNA feedback circuit in midbrain dopamine neurons. *Science*. 2007;317(58):1220–1224. doi:10.1126/science.1140481
41. Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. *Methods Mol Biol*. 2017;1509(3):1–10.
42. Hayes J, Peruzzi P, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med*. 2014;20(8):460–469. doi:10.1016/j.molmed.2014.06.005
43. Bratosin D, Estaquier J, Petit F, et al. Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. *Cell Death Differ*. 2001;8(12):1143–1156. doi:10.1038/sj.cdd.4400946
44. Chen Y, Wang Y, Telen J, Chi T. The genomic analysis of erythrocyte microRNA expression in sickle cell diseases. *PLoS One*. 2008;3(6):23–60.
45. Kannan M, Atreya C. Differential profiling of human red blood cells during storage for 52 selected microRNAs. *Transfusion*. 2010;50(7):1581–1588. doi:10.1111/j.1537-2995.2010.02585.x
46. Flatt F, Bawazir M, Bruce J. The involvement of cation leaks in the storage lesion of red blood cells. *Front Physiol*. 2014;5:214. doi:10.3389/fphys.2014.00214
47. Yang H, Doss F, Walzer A, et al. Angiogenin-mediated tRNA cleavage as a novel feature of stored red blood cells. *Br J Haematol*. 2019;185(4):760–764. doi:10.1111/bjh.15605
48. Azzouzi I, Moest H, Wollscheid B, Schmutz M, Eekels M, Speer O. Deep sequencing and proteomic analysis of the microRNA-induced silencing complex in human red blood cells. *Exp Hematol*. 2015;43(5):382–392. doi:10.1016/j.exphem.2015.01.007
49. Chen P, Hong J, Chi J. Discovery, genomic analysis, and functional role of the erythrocyte RNAs. *Curr Pathobiol Rep*. 2017;5(1):43–48. doi:10.1007/s40139-017-0124-z
50. Jian F, Peng Y, Bian M. Expression and bioinformatics analysis of key miRNAs in stored red blood cells. *Transfus Med Hemother*. 2022;21(3):1–8.
51. Andreasen D, Fog U, Biggs W, et al. Improved microRNA quantification in total RNA from clinical samples. *Blood Transfus*. 2010;50(4):6–9.
52. Zhang Y, Huang G, Yuan Z, et al. Profiling and bioinformatics analysis revealing differential circular RNA expression about storage lesion regulatory in stored red blood cells. *Transfus Med Hemother*. 2022;49(2):76–87. doi:10.1159/000519626
53. Sangokoya C, LaMonte G, Chi T. Isolation and characterization of microRNAs of human mature erythrocytes. *Methods Mol Biol*. 2010;667(3):193–203.
54. Kannan M, Kulkarni S, Atreya C. Apoptotic microRNA profiling of packed red blood cells during storage. *Blood*. 2009;114(22):3145–3149. doi:10.1182/blood.V114.22.3145.3145
55. Sarachana T, Kulkarni S, Atreya D. Evaluation of small noncoding RNAs in ex vivo stored human mature red blood cells: changes in noncoding RNA levels correlate with storage lesion events. *Transfusion*. 2015;55(11):2672–2683. doi:10.1111/trf.13235

56. Haberberger A, Kirchner B, Riedmaier I, et al. Changes in the microRNA expression profile during blood storage. *BMJ Open Sport Exer Med*. 2018;4(1):354–360. doi:10.1136/bmjsem-2018-000354
57. Kong Y, Tian X, He R, et al. The accumulation of exosome-associated microRNA-1246 and microRNA-150-3p in human red blood cell suspensions. *J Transl Med*. 2021;19(1):225–228. doi:10.1186/s12967-021-02887-2
58. Chen X, Xie X, Xing Y, Yang X, Yuan Z, Wei Y. MicroRNA dysregulation associated with red blood cell storage. *Transfus Med Hemother*. 2018;45(6):397–402. doi:10.1159/000489321
59. Huang H, Zhu J, Fan L, et al. MicroRNA profiling of exosomes derived from red blood cell units: implications in transfusion-related immunomodulation. *Biomed Res Int*. 2019;19(2):459–465.
60. Zare S, Mousavi K, Maghsudlu M, Shahabi M. miRNA96 expression level within red blood cells is probably associated with RSL indicators during the storage of red blood cell units. *Transfus Apher Sci*. 2021;60(3):103–122. doi:10.1016/j.transci.2021.103122
61. Vu L, Ragupathy V, Kulkarni S, Atreya C. Analysis of Argonaute 2-microRNA complexes in ex vivo stored red blood cells. *Transfusion*. 2017;57(12):2995–3000. doi:10.1111/trf.14325
62. Chen CZ, Li L, Lodish F, Bartel P. MicroRNAs modulate hematopoietic lineage differentiation. *Science*. 2004;303(5654):83–86. doi:10.1126/science.1091903
63. Linsley P, Schelter J, Burchard J, et al. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol Cell Biol*. 2007;27(6):2240–2252. doi:10.1128/MCB.02005-06
64. Yan X, Liang H, Deng T, et al. The identification of novel targets of miR-16 and characterization of their biological functions in cancer cells. *Mol Cancer*. 2013;12(5):92–95. doi:10.1186/1476-4598-12-92
65. Wulczyn G, Smirnova L, Rybak A, et al. Post-transcriptional regulation of the let-7 microRNA during neural cell specification. *FASEB j*. 2007;21(2):415–426. doi:10.1096/fj.06-6130com
66. Patrick M, Zhang C, Tao Y, et al. Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3zeta. *Genes Dev*. 2010;24(15):1614–1619. doi:10.1101/gad.1942810
67. Rasmussen D, Simmini S, Abreu-Goodger C, et al. The miR-144/451 locus is required for erythroid homeostasis. *J Exp Med*. 2010;207(7):1351–1358. doi:10.1084/jem.20100458
68. Bolandghamat Pour Z, Nourbakhsh M, Mousavizadeh K, et al. Up-regulation of miR-381 inhibits NAD⁺ salvage pathway and promotes apoptosis in breast cancer cells. *Excli J*. 2019;18(6):683–696. doi:10.17179/excli2019-1431
69. Liao W, Zhang Y. MicroRNA-381 facilitates autophagy and apoptosis in prostate cancer cells via inhibiting the RELN-mediated PI3K/AKT/mTOR signaling pathway. *Life Sci*. 2020;254(4):117–122. doi:10.1016/j.lfs.2020.117672
70. Wan Y, Cui R, Gu J, et al. Identification of four oxidative stress-responsive microRNAs, miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p, in hepatocellular carcinoma. *Oxid Med Cell Longev*. 2017;2017(1):189–198. doi:10.1155/2017/5189138
71. Luo E, Chang Y, Sher Y, et al. MicroRNA-769-3p down-regulates NDRG1 and enhances apoptosis in MCF-7 cells during reoxygenation. *Sci Rep*. 2014;4(2):590–594. doi:10.1038/srep05908
72. Bhagirath D, Yang T, Bucay N, et al. microRNA-1246 is an exosomal biomarker for aggressive prostate cancer. *Cancer Res*. 2018;78(7):1833–1844. doi:10.1158/0008-5472.CAN-17-2069
73. Yang Y, Hou Y, Li J, Zhang F, Du Q. Characterization of antiapoptotic microRNAs in primary Sjögren's syndrome. *Cell Biochem Funct*. 2020;38(8):1111–1118. doi:10.1002/cbf.3569
74. Meerson A, Yehuda H. Leptin and insulin up-regulate miR-4443 to suppress NCOA1 and TRAF4, and decrease the invasiveness of human colon cancer cells. *BMC Cancer*. 2016;16(1):882–885. doi:10.1186/s12885-016-2938-1
75. Le M, Xie H, Zhou B, et al. MicroRNA-125b promotes neuronal differentiation in human cells by repressing multiple targets. *Mol Cell Biol*. 2009;29(19):5290–5305. doi:10.1128/MCB.01694-08
76. Luo E, Chang Y, Sher Y, et al. MicroRNA-769-3p down-regulates NDRG1 and enhances apoptosis in MCF-7 cells during reoxygenation. *Sci Rep*. 2014;4(1):5908–5911.
77. Liu H, Zhang Y, Zhu B, et al. MiR-203a-3p regulates the biological behaviors of ovarian cancer cells through mediating the Akt/GSK-3 β /Snail signaling pathway by targeting ATM. *J Ovarian Res*. 2019;12(1):60–65. doi:10.1186/s13048-019-0532-2
78. Xiong J, Xing S, Dong Z, et al. miR-654-3p suppresses cell viability and promotes apoptosis by targeting RASAL2 in non-small-cell lung cancer. *Mol Med Rep*. 2021;23(2):78–82. doi:10.3892/mmr.2020.11721
79. Zhang H, Li H, Wang G, et al. MKL1/miR-5100/CAAP1 loop regulates autophagy and apoptosis in gastric cancer cells. *Neoplasia*. 2020;22(5):220–230. doi:10.1016/j.neo.2020.03.001
80. de Gonzalo-Calvo D, Pérez-Boza J, Curado J, Devaux Y. Challenges of microRNA-based biomarkers in clinical application for cardiovascular diseases. *Clin Transl Med*. 2022;12(2):585–587.
81. Vickers K, Palmisano B, Shoucri B, Shamburek R, Remaley A. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol*. 2011;13(4):423–433. doi:10.1038/ncb2210
82. Jian F, Peng Y, Bian M. Expression and bioinformatics analysis of key miRNAs in stored red blood cells. *Transfus Med Hemother*. 2022;30(3):1–8.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Anticoagulation Management in Patients Receiving Warfarin at Private Cardiac Centers in Addis Ababa, Ethiopia

Roza Getachew, Tamrat Assefa Tadesse, Bekele Alemayehu Shashu, Amsalu Degu & Getachew Alemkere

To cite this article: Roza Getachew, Tamrat Assefa Tadesse, Bekele Alemayehu Shashu, Amsalu Degu & Getachew Alemkere (2023) Anticoagulation Management in Patients Receiving Warfarin at Private Cardiac Centers in Addis Ababa, Ethiopia, Journal of Blood Medicine, , 107-117, DOI: [10.2147/JBM.S397189](https://doi.org/10.2147/JBM.S397189)

To link to this article: <https://doi.org/10.2147/JBM.S397189>



© 2023 Getachew et al.



Published online: 10 Feb 2023.



Submit your article to this journal



Article views: 379



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Anticoagulation Management in Patients Receiving Warfarin at Private Cardiac Centers in Addis Ababa, Ethiopia

Roza Getachew¹, Tamrat Assefa Tadesse¹, Bekele Alemayehu Shashu², Amsalu Degu³, Getachew Alemkere¹

¹Department of Pharmacology and Clinical Pharmacy School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia;

²Department of Internal Medicine School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; ³Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy & Health Sciences, United States International University Africa, Nairobi, Kenya

Correspondence: Tamrat Assefa Tadesse, Email tamrat.assefa@aau.edu.et

Introduction: Anticoagulants are the cornerstone therapy for thromboembolism prevention and treatment. Warfarin is the frequently prescribed drug and remains the oral anticoagulant of choice in low- and middle-income countries, including Ethiopia. It is a narrow therapeutic index drug that needs high-quality anticoagulation monitoring with frequent international normalization ratio (INR) testing.

Objective: The study aimed to assess anticoagulation management with warfarin among adult outpatients at two selected private cardiac centers in Addis Ababa, Ethiopia.

Methods: A hospital-based retrospective study design that enrolled 374 patients receiving warfarin was employed at two private cardiac centres in Addis Ababa, Ethiopia. The time in the therapeutic range (TTR) was calculated using the Rosendaal method. The data were analyzed using Statistical Package for Social Science version 25.

Results: The mean age of the patients was 57 years, and 218 (58.3%) participants were females. Out of 3384 INR tests, 1562 (46.5%) were within the therapeutic range and the mean percentage of TTR was 47.24%. Only 25.67% of the patients spent their TTR $\geq$ 65%. The present study revealed that dose adjustments were required 1764 times. In non-therapeutic INR values of 1764 that required warfarin dose adjustment, 59.7% of the doses were adjusted. About 262 (70.1%) of co-prescribed medications had interaction with warfarin. Sixty-four patients (17.11%) experienced bleeding events.

Conclusion: Anticoagulation management with warfarin was suboptimal in private cardiac Addis Ababa, Ethiopia, private cardiac centers. Warfarin adjustment practice for nontherapeutic INR values was not minimal, and many patients encountered bleeding during their course of therapy.

Keywords: warfarin, anticoagulation, time in the therapeutic range, international normalization ratio, Ethiopia

Introduction

The safety and efficacy of warfarin therapy depend on careful monitoring and maintenance of the international normalized ratio (INR) within an optimal therapeutic range.¹ Its narrow therapeutic range, frequent drug, herbal, and food interactions, and the effect of comorbidities place patients at risk of bleeding and thromboembolic complications if the recommended anticoagulation target ranges are not achieved.^{2–7} Furthermore, factors related to drugs, patients, and healthcare will compromise the overall quality of anticoagulation management with warfarin therapy which is reflected by actual time spent within the therapeutic INR, ie, time in the therapeutic range (TTR).^{3,8–10} The TTR estimates the percentage of time a patient's INR is within the desired treatment range or goal and is widely used as an indicator of anticoagulation control.¹¹

A minimum TTR of 65% is required for warfarin therapy to be regarded as effective,^{12,13} and below this value, warfarin is unlikely to prevent thromboembolic disease effectively. In another way, the risk of both thromboembolism and bleeding has also been shown to depend on the percentage of INR in the therapeutic range.¹⁴ However, in the real

world, maintaining adequate anticoagulation with warfarin has proved to be challenging due to the complexity of warfarin therapy. This was demonstrated by studies conducted globally with low TTR (<65%).^{1,11,15–23} The problem seems the worst in Africa, as the lowest TTRs were reported in different regions of the continent.^{7,20,24–27} Similarly, in Ethiopia, only four studies were conducted in government health facilities and reported lower TTRs in the range of 29–38%.^{28–31} Despite the wide availability of warfarin as an oral anticoagulant, information on its level of anticoagulation control is scarce in Ethiopia, especially in private settings. Therefore, this study aimed to determine the proportion of time spent in the therapeutic INR range and investigate dose adjustment practice in patients taking warfarin in selected private health facilities in Addis Ababa, Ethiopia.

Methods

Study Setting

The study was conducted at two private cardiac centers in Addis Ababa, Ethiopia, namely Gesund Cardiac and Medical Center (GCMC) and Addis Cardiac Hospital (ACH). GCMC is a 31-bed cardiac center; annually, it serves around 41,500 patients and about 2000 of them receive warfarin. ACH is Ethiopia's first specialized cardiovascular hospital with advanced investigations and treatment modalities for cardiac diseases, including heart surgery, implantable devices, and percutaneous coronary intervention. The hospital has 43 beds and serves 51,700 patients annually. Among these, 1683 patients were receiving warfarin therapy during our study period.

Study Design and Period

A retrospective study was carried out at ACH and GCMC from 1 February to 30 April 2021 on patients receiving warfarin.

Source and Study Population

All adult patients receiving warfarin for 3 months and above for various indications at study centers were the source population. All eligible patients on warfarin who had follow-ups at ACH and GCMC from 1 January 2019 to 31 December 2020 (2 years) were considered as the study population.

Sample Size Determination and Sampling Techniques

The sample size was determined using the single population proportion formula,³² ie,

$$n = \frac{Z_{\alpha/2}^2 \times P(1 - P)}{d^2} \quad n = \frac{Z_{\alpha/2}^2 \times P(1 - P)}{d^2} = \frac{(1.96)^2 \times 0.3791(1 - 0.3791)}{(0.05)^2} = 361.70 = \sim 362$$

where n = minimum sample size, Z = standard normal distribution ($Z_{\alpha/2}=1.96$) with 95% confidence interval, d = absolute precision or tolerable margin of error (d) = 5% (0.05) and P = prevalence is essential proportion). From a recent previous study in Northern Ethiopia, a proportion of 0.3791, ie, a mean TTR of 37.91%³³ was used. With a 10% contingency to cater to incomplete medical records, the final sample size was 398. Finally, we reviewed 374 patients' charts (187 from each site) that fulfilled the inclusion criteria during the study period. A 2-year list of hospital identification numbers of warfarin-treated patients was generated from the health record. The actual sampling fraction (kth) was calculated by dividing the total number of the study population attending the hospital during the study period (3671) by the estimated sample size of the study. Thus, approximately every 9 patients' chart was reviewed.

Eligibility Criteria

Adult patients (≥ 18 years) who had been on warfarin for ≥ 3 months with two or more INR values were included in the study. Nonetheless, medical records of patients with incomplete information about the INR values and other pertinent findings were excluded from the study.

Data Collection Instruments and Techniques

The data abstraction instrument was prepared by reviewing different literature. The data were collected by three pharmacists and one nurse after providing relevant training. Then, all the required pieces of information such as demographic characteristics, indications of anticoagulation, comorbidities, drugs prescribed with warfarin, and corresponding INR results were recorded by reviewing the medical records of the patients. The data abstraction tool had been pretested on 5% of the study participants before actual data collection. All relevant amendments were made after pretesting the data abstraction tool. Percentages of TTR were calculated using the Rosendaal method,³⁴ and the mean frequency of INR values and percentage of warfarin dose adjusted were determined.

Data Analysis and Interpretation

Data were analyzed using Statistical Package for Social Science (SPSS) version 25.0 software. Socio-demographic and clinical characteristics were summarized by percentages and frequencies. Micromedex Healthcare Series [online database] Version 5.1³⁵ was used to analyze drug interactions with warfarin.

Ethical Considerations

Ethical clearance was obtained from the Ethics Review Committee of the School of Pharmacy, College of Health Sciences of Addis Ababa University (ERB/SOP/244/13/2021). This study complies with the guidelines of the Declaration of Helsinki. Confidentiality of the information was maintained by avoiding the use of individual identifiers like names and the patients' hospital identification numbers.

Results

The mean age of the study participants was 57 ± 18 years, with an age range of 18–92 years. The majority of the study participants were female (218, 58.3%). Heart failure (36.89%) and hypertension (17.2%) were the most common comorbidities in patients receiving warfarin in this study. Polypharmacy was seen in 51.34% of the patients (Table 1).

Indication of Warfarin

The most common indication of warfarin was for atrial fibrillation patients, followed by chronic rheumatic valvular heart disease (Figure 1).

INR Distribution

During 2-year follow-up period, of 374 patients, 88.51% and 11.49% had target INR ranges of 2–3 and 2.5–3.5 with 1477 and 85 INR values within respective target ranges, respectively. The average number of INR tests per patient was 4. The detailed INR distribution is shown in Figure 2.

Time in the Therapeutic Range

According to the Rosendaal method, the mean percentages of TTR were 47.24% (Figure 3), and only 25.67% of the patients spent their time above TTR of 65%.

Warfarin Dose Adjustment

This study revealed that dose adjustments were required 1764 times for nontherapeutic INR values. However, it was only adjusted 1053 times (873 for sub-therapeutic and 180 times for the supratherapeutic range). The details for warfarin dose adjustment for non-therapeutic INR values are given in Table 2.

Drug–Drug Interactions Among Patients Taking Warfarin

About 262 (70.0%) patients were prescribed medications with the potential of interacting with warfarin. Furosemide (89.84%), spironolactone (72.9%) and metoprolol succinate (63.63%) were the most repeatedly observed drugs that

Table 1 Socio-Demographic and Clinical Characteristics of Patients

Variables	Category	GCMC	ACH	Total
		n(%)	n(%)	n(%)
Age in years	18–40	29(15.5)	38(20.3)	67(17.91)
	41–64	78(41.7)	74(39.6)	152(40.64)
	65–74	48(25.7)	51(27.3)	99(26.47)
	≥75	32(17.1)	24(12.8)	56(14.97)
Sex	Female	110(58.8)	108(57.8)	218(58.3)
	Male	77(41.2)	79(42.2)	156(41.7)
Comorbid conditions	Heart failure	65(34.8)	69(36.89)	134(35.83)
	Hypertension	32(17.2)	29(15.5)	61(16.3)
	Hypertension+ hyperthyroidism	14(7.5)	12(6.4)	26(6.95)
	Diabetic mellitus	9(4.8)	5(2.7)	14(3.74)
	Hypertension + heart failure	8(4.3)	4(2.1)	12(3.2)
	Hypertension + chronic renal failure	6(3.2)	2(1.1)	8(2.14)
	Hyperthyroidism	5(2.7)	4(2.1)	9(2.4)
	Hypertension+ diabetic mellitus + heart failure	5(2.7)	6(3.2)	11(2.94)
	Chronic renal failure	4(2.1)	3(1.6)	7(1.87)
	Hypertension + heart failure + hyperthyroidism	4(2.1)	3(1.6)	7(1.87)
	Hypertension+ diabetic mellitus + heart failure+ hyperthyroidism	3(1.6)	2(1.1)	5(1.34)
	Others*	12(6.4)	18(9.62)	30(8.02)
Number of medications prescribed per patient	1–2	18(9.6)	24(12.1)	42(11.23)
	3–4	54(28.9)	86(43.4)	140(37.43)
	≥5	115(61.5)	77(38.1)	192(51.34)

Notes: *Include infectious disease, renal disorder, cancer, depression, hypothyroidism, HIV/AIDS, anemia, arthritis, Asthma, coronary obstructive pulmonary disease. n: frequency.

Abbreviations: GCMC, Gesund Cardiac and Medical Center; ACH, Addis Cardiac Hospital.

interact with warfarin in the present study. Only major and moderate interactions were reported based on the Micromedex interaction checker (Table 3).

Bleeding Events

In this study, 64 (17.11%) patients encountered minor and major bleeding events. Of these, 42 patients experienced minor bleeding events (nasal and vaginal bleeding) with therapeutic INR value. Seven patients had INR values of 3.1–4.99, while 11 patients had INR values greater than 5, and 4 patients experienced intracranial hemorrhages with an INR value of >5.

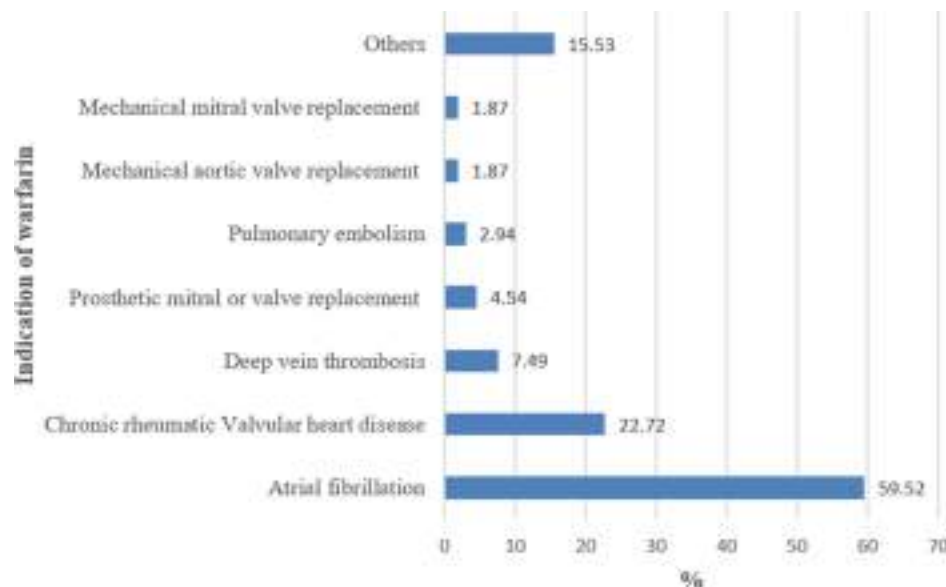


Figure 1 Indication of warfarin therapy.

Discussion

This study aimed to assess anticoagulation management with warfarin at private health facilities in Addis Ababa, Ethiopia. The majority of the study participants (58.3%) were females, and the mean age was 57 years. This female predominance was comparable to the study done in Kenya,³⁶ and Ethiopia.^{29,37,38} In contrast, other studies conducted in Poland³⁹ and Canada¹⁶ reported male predominance. Atrial fibrillation (43.85%) was the commonest indication for warfarin therapy in the current study.

The duration of time a patient is on oral anticoagulant influences the stability of INR. Moreover, dietary habits and the presence of drug interaction may alter INR levels associated with warfarin treatment.⁴⁰ This study revealed that the mean frequency of INR monitoring per patient was every 47.85 days. The frequency of INR monitoring obtained in this study (every 47.85 days) was more infrequent when compared with the study done in western Ethiopia, where most patients (84%) had INR determined every month.³⁷ In addition, a study from Kenya showed that the median frequency of INR monitoring was 18.5 days.³⁶ Another study in Ethiopia reported the interval between two INR tests was 35 days.⁴¹ Comparatively, there was more frequent monitoring of INR in the study settings as compared to reports from a tertiary care teaching hospital in Ethiopia (64 days per patient).³⁸ These disparities could probably be linked to the difference in the patient size population and specific trends in the management of patients receiving warfarin therapy in the study settings, which can affect their capacity to monitor INR frequently. Furthermore, it is also noticed that warfarin monitoring is patient-tailored. However, current guidelines suggest that at least a 4-week monitoring is essential. This time should be reduced to a few days on several occasions including in the case of frequent non-therapeutic INRs and the occurrence of bleeding and thrombotic events.

The TTR (47.24%) obtained in this study was higher than reports from Lithuania (40%),⁴² Uganda (41%),⁴³ Spain (25.9%),⁴⁴ Ethiopia (29%),³⁸ Pakistan (34.9%),⁴⁵ Botswana (30.8%),⁴⁶ and Northern Ethiopia (33%).³⁷ However, far away from recommended optimal TTR ($\geq 70\%$), optimal anticoagulation in patients receiving warfarin⁴⁷ and also low as compared to previous studies in New Zealand (63%)⁴⁸ and Brazil (56.6%).⁴⁹ This difference could probably be the higher level of health care in developing countries that had better anticoagulation monitoring services. In addition, this poor TTR in the study setting may be due to infrequent INR monitoring and a lack of separate anticoagulation clinics. The present study showed that 1764 INR tests were deemed to have warfarin dose adjustment. Nonetheless, doses were adjusted in only 1053 (59.69%) values. This can probably be linked to physicians' adherence problems, lack of functional

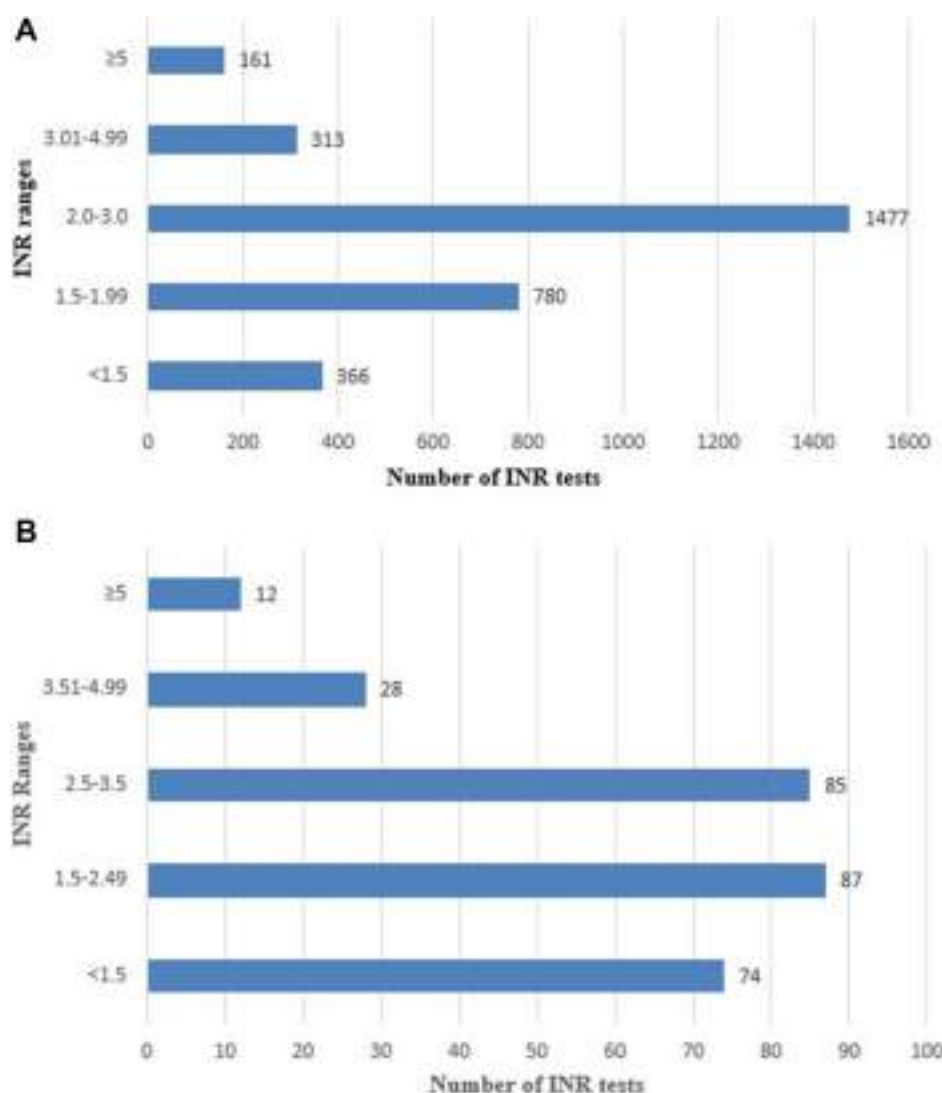


Figure 2 INR values distributions within different intervals among patients taking warfarin for achieving target ranges (A :2.0–3.0, B: 2.5–3.5).

dose adjustment algorithm, warfarin drug–drug interaction, and drug–food interactions that affect warfarin serum levels. However, in the study settings, the management of nontherapeutic (sub-therapeutic) INR was better than in previous studies conducted in government hospitals in Ethiopia,^{29,38} although the management of nontherapeutic INR value was not satisfactory.

The most commonly prescribed warfarin-interacting drugs were furosemide, followed by spironolactone and metoprolol succinate in the study settings. The prevalence of drug–drug interactions with warfarin was found to be 70%. This result was comparable with a study conducted in Thailand that identified 71% warfarin drug interaction,¹ but it is better than that of Ayder Referral Hospital, Ethiopia (99.2%).⁵⁰ Other studies at Tertiary Care Hospitals in Ethiopia identified a lower percentage of warfarin drug interaction.^{41,51} This high percentage of drug–drug interaction with warfarin may be justified by the high prevalence of co-prescribed interacting medications.

The most common complication of warfarin therapy was bleeding. This study reveals 64 (17.11%) minor and major (intracranial hemorrhage) bleeding events, which were lower in incidence as compared with studies from Ethiopia (25%) and Thailand (32.56%).^{52,53} However, a retrospective study in Brazil reported comparable bleeding events (4.2% major and 10.3% minor bleeding episodes) with the present study.⁴⁹

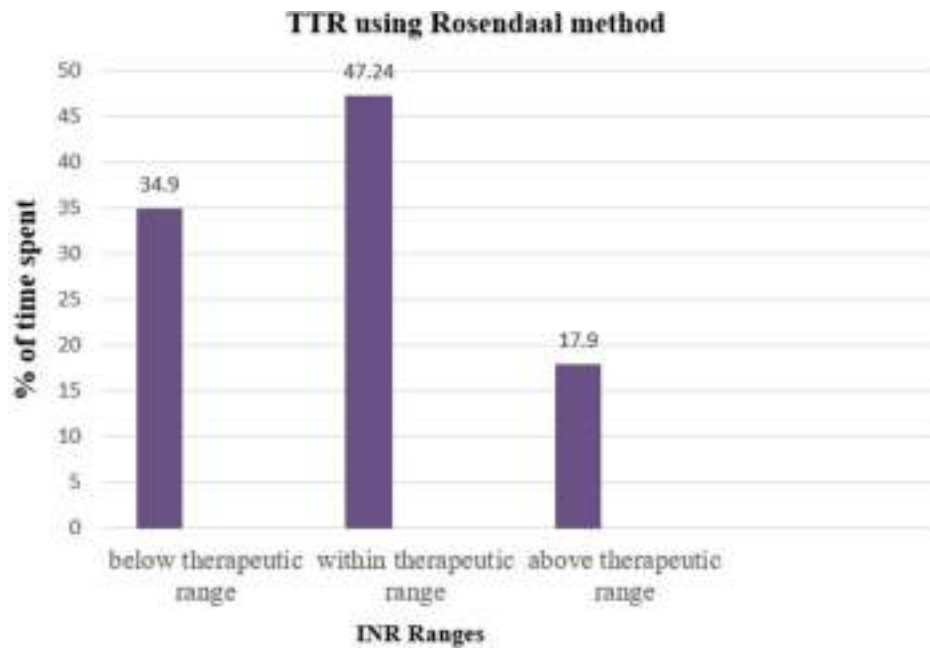


Figure 3 Time spent in different INR ranges in patients taking warfarin.

Limitations of the Study

Due to the retrospective nature of the study, the data will be affected by the completeness and accuracy of the information in the study settings. Therefore, it is difficult to clarify some problems in definite detail, such as the cause of non-adherence or the type of herbs that interact with warfarin, factors that may affect anticoagulation like adherence, diet, physical activity, and genetic variability. There was a risk of information bias as this retrospective study relied on information from patient files. It is also prone to missing patient information if they had sought other health facilities that were not documented.

Table 2 Warfarin Dose Adjustment Practice for Non-Therapeutic INRs in Patients Receiving Warfarin

Non-therapeutic NRs	Dose Decreased(%)	Dose Increased(%)	Not Adjusted(%)
Subtherapeutic (1310)	213(16.26)	873(66.64)	224(17.09)
Supra-therapeutic (454)	180(39.65)	77(16.96)	197(43.39)

Table 3 Warfarin Drug Interactions Among Patients Taking Warfarin

Types of Drug Interaction	Co-Prescribed Drugs	Frequency(%)
Major	Furosemide	336(89.84)
	Spironolactone	273(72.9)
	Metoprolol succinate	238(63.63)
	Digoxin	153(40.9)
	Enalapril	133(35.56)

(Continued)

Table 3 (Continued).

Types of Drug Interaction	Co-Prescribed Drugs	Frequency(%)
	Atorvastatin	124(33.15)
	Atenolol	113(30.21)
	Bisoprolol	85(22.72)
	Amiodarone	65(17.38)
	Amlodipine	60(16.04)
	Aspirin	58(15.5)
	Metformin	49(13.1)
	Carvedilol	41(10.96)
	Amoxicillin plus clavulanic acid	25(6.68)
	Lisinopril	21(5.6)
	Benzathine penicillin	21(5.6)
	Rosuvastatin	19(5.08)
	Hydrochlorothiazide	18(4.8)
	Azithromycin	15(8.02)
	Cefixime	12(3.2)
	Norfloxacin	4(2.14)
Moderate	Omeprazole	29(7.7)
	Propylthiouracil	26(6.95)
	Others	27(7.2)

Notes: Others; Salbutamol puff, Candesartan/hydrochlorothiazide, Haem Up syrup, acyclovir. Others include double aortic and mitral prosthetic valve replacement, dilated cardiomyopathy, old stroke and post-myocardial infarction.

Conclusion

Anticoagulation management with warfarin was suboptimal in the study settings, which can significantly affect the desired treatment outcomes of the patients. In addition, most of the co-prescribed medications had undesired drug–drug interactions with warfarin and need attention. Therefore, optimal interventional strategies are required to improve the treatment outcomes of patients receiving warfarin therapy.

Abbreviations

ACH, Addis Cardiac Hospital; AF, atrial fibrillation; DVT, deep vein thrombosis; INR, GCMC, Gesund Cardiac and Medical Center; INR, international normalization ratio; SPSS, Statistical Package for Social Science; TTR, time in therapeutic range; VTE, venous thromboembolism.

Data Sharing Statement

The datasets used in this study are available from the corresponding author upon request.

Declarations Ethics Approval and Consent to Participate

This study was approved by the Ethical Review committee of the School of Pharmacy, College of Health Sciences Addis Ababa University, Addis Ababa, Ethiopia (ERB/SOP/244/13/2021). Then, a support letter was written by the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy to GCMC and ACH, and permission was obtained from the study settings to conduct the study. Information obtained from data collected during the study was only handled by the research team. Personal identifiers were not used, and data were analyzed in aggregate. The Ethics Committee waived the need for informed consent since this was an observational study using existing data.

Acknowledgments

The authors would like to acknowledge the staff members of ACH and GCMC healthcare facilities for their unreserved support during the entire data collection process for this project.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

References

1. Albabtain MA, Alharthi MM, Dagriri K., et al. Assessment of the quality of anticoagulation management with warfarin in a tertiary care center. *Saudi Med J*. 2020;41(11):1245–1251. doi:10.15537/smj.2020.11.25456
2. Laàs DJ, Naidoo M. An evaluation of warfarin use at an urban district-level hospital in KwaZulu-Natal Province, South Africa. *South Af Med J*. 2018;108(12):1046–1050. doi:10.7196/SAMJ.2018.v108i12.13256
3. Witt DM, Nieuwlaar R, Clark NP, et al. American Society of Hematology 2018 guidelines for the management of venous thromboembolism: optimal management of anticoagulation therapy. *Blood Adv*. 2018;2(22):3257–3291. doi:10.1182/bloodadvances.2018024893
4. Zeitler EP, Eapen ZJ, Clinical D, Ne D. Anticoagulation in heart failure: a review. *J Atrial Fibrillation*. 2015;8(1). doi:10.4022/jafib.1250
5. Di MA, Frigerio B, Spadarella G, et al. Old and new oral anticoagulants: food, herbal medicines and drug interactions. *Blood Rev*. 2017;31(4):193. doi:10.1016/j.blre.2017.02.001
6. Fibrillation A, Nelson WW, Choi JC, et al. Impact of co-morbidities and patient characteristics on international normalized ratio control over time in patients with nonvalvular. *Am J Cardiol*. 2013;112(4):509–512. doi:10.1016/j.amjcard.2013.04.013
7. Karuri S, Nyamu D, Opanga S, Menge T. Factors associated with time in therapeutic range among patients on oral anticoagulation therapy in a tertiary teaching and referral hospital in Kenya. *East Cent Af J Pharm Sci*. 2019;22(3):85–95.
8. Alghadeer S, Alzahrani AA, Alalayet WY, Alkharashi AA, Alarifi MN. Anticoagulation control of warfarin in pharmacist-led clinics versus physician-led clinics: a prospective observational study. *Risk Manag Healthc Policy*. 2020;13:1175–1179. doi:10.2147/RMHP.S248222
9. Mwita JC, Francis JM, Oyekunle AA, Gaenamang M, Goepamang M, Magafu MGD. Quality of anticoagulation with warfarin at a tertiary hospital in Botswana. *Clin Appl Thrombosis Hemostasis*. 2018;24(4):596–601. doi:10.1177/1076029617747413
10. Gabriel P, De BM, Szejder H, et al. Anticoagulation therapy in patients with non-valvular atrial fibrillation in a private setting in Brazil: a real-world study. *Arq Bras Cardiol*. 2020;114:457.
11. Farsad B, Abbasnazar M, Dabagh A, Bakshandeh H. Evaluation of time in therapeutic range (TTR) in patients with non-valvular atrial fibrillation receiving treatment with warfarin in Tehran, Iran: a cross-sectional study. *J Clin Diagnostic*. 2016;20(9):20–22. doi:10.7860/JCDR/2016/21955.8457
12. Prinsloo DN, Gould TJ, Viljoen CA, Basera W, Ntsekhe M. International normalised ratio control in a non-metropolitan setting in Western Cape Province, South Africa. *South African Med J*. 2021;111(4):355–360. doi:10.7196/SAMJ.2021.v111i4.15171
13. Pastori D, Farcomeni A, Saliola M, Del F, Pignatelli P. Temporal trends of time in therapeutic range and incidence of cardiovascular events in patients with non-valvular atrial fibrillation. *Eur J Intern Med*. 2018;1. doi:10.1016/j.ejim.2018.04.007
14. Ntlokotsi S, Moshesh MF, Mntla P, Towobola OA, Mogale MA. Optimum INR intensity and therapeutic INR control in patients with mechanical heart valve prosthesis on warfarin oral anticoagulation at Dr George Mukhari academic hospital: a three-year retrospective study. *South African Family*. 2018;60(6):192–196.
15. Caldeira D, Cruz I, Morgado G, et al. Evaluation of time in therapeutic range in anticoagulated patients: a single-center, retrospective, observational study. *Revista Portuguesa de Cardiologia*. 2014;34(1):1–5. doi:10.1016/j.repc.2014.08.005
16. Gateman D, Trojnar ME, Agarwal G, Gateman D, Trojnar ME, Agarwal G. Time in therapeutic range Un RIN dans la fourchette thérapeutique. *Canadian Family Phys*. 2017;63:425–431.
17. Chan P. Time in therapeutic range and percentage of inrs in therapeutic range as measure of quality of anticoagulation control in atrial fibrillation patients. *Can J Cardiol*. 2015;32(10):1247.e23–1247.e28. doi:10.1016/j.cjca.2015.10.029

18. Han SY, Palmeri ST, Broderick SH, et al. Quality of anticoagulation with warfarin in patients with nonvalvular atrial fibrillation in the community setting. *J Electrocardiol.* 2013;46(1):45–50. doi:10.1016/j.jelectrocard.2012.08.011
19. Botsile E, Mwita JC. Incidence and risk factors for thromboembolism and major bleeding in patients with mechanical heart valves: a tertiary hospital-based study in Botswana. *Cardiovasc J Afr.* 2020;31(4):185–189. doi:10.5830/CVJA-2020-006
20. Jonkman LJ, Gwanyanya MP, Kakololo MN, Verbeeck RK, Singu BS. Assessment of anticoagulation management in outpatients attending a warfarin clinic in Windhoek, Namibia. *Drugs Ther Perspect.* 2019;35(7):341–346. doi:10.1007/s40267-019-00630-y
21. Ugur A, Turk O, Tuncer E, Alioglu E, Yuksel K. Evaluation of the impact of warfarin s time-in-therapeutic range on outcomes of patients with atrial fibrillation in Turkey: perspectives from the Observational, Prospective WATER Registry. *Cardiology J.* 2015;1:548. doi:10.5603/CJ.a2015.0035
22. Quinn LM, Richardson R, Cameron KJ, Battistella M. Evaluating time in therapeutic range for hemodialysis patients taking warfarin. *Clin Nephrol.* 2015;83(2):80–85. doi:10.5414/CN108400
23. de Castro KP. A Patient decision aid for anticoagulation therapy in patients with nonvalvular atrial fibrillation: development and pilot study. *JMIR Cardio.* 2021;5(2):e23464. doi:10.2196/23464
24. Sonuga BO, Hellenberg DA, Cupido CS, Jaeger C. Profile and anticoagulation outcomes of patients on warfarin therapy in an urban hospital in Cape Town, South Africa. *Af J Prim Heal Care Fam Med.* 2016;8(1):e1–e8. doi:10.4102/phcfm.v8i1.1032
25. Ouali S, Ben Halima A, Chabrak S, et al. Epidemiological characteristics, management, and outcomes of atrial fibrillation in Tunisia: results from the National Tunisian Registry of Atrial Fibrillation (NATURE-AF). *Clin Cardiol.* 2021;44(4):501–510. doi:10.1002/clc.23558
26. Mariita K, Nyamu DG, Maina CK, Karimi PN. Patient factors impacting on oral anticoagulation therapy among adult outpatients in a Kenyan referral hospital. *Af J Pharmacol Therapeutics.* 2016;1:548.
27. Sadhabariss D, Brown SL. Warfarin: time in therapeutic range, a single centre study on patients using warfarin for stroke prevention in non-valvular atrial fibrillation and prosthetic heart valves. *SA Hear.* 2021;18(1):28–38. doi:10.24170/18-1-4771
28. Fenta TG, Assefa T, Alemayehu B. Quality of anticoagulation management with warfarin among outpatients in a tertiary hospital in Addis Ababa, Ethiopia: a retrospective cross-sectional study. *Af J Pharmacol.* 2017;1–7. DOI:10.1186/s12913-017-2330-0
29. Yimer NS, Abiye AA, Hussien SU, Tadesse TA. Anticoagulation control, outcomes, and associated factors in patients with atrial fibrillation receiving warfarin at tertiary care hospital in Ethiopia. *Clin Appl Thromb.* 2021;27:107602962110497. doi:10.1177/10760296211049786
30. Liyew Z, Tadesse A, Bekele N, Tsegaye T. Evaluation of anticoagulation outcome among patients taking warfarin: a single-center experience, Northwest Ethiopia. *Res Sq.* 2017;20–25.
31. Masresha N, Muche EA, Atnafe A, Abdela O. Evaluation of warfarin anticoagulation at University of Gondar comprehensive specialized. *J Blood Med.* 2021;12:189–195. doi:10.2147/JBM.S282948
32. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Bench.* 2013;6(1):14–17.
33. Masresha N, Muche EA, Atnafe A, Abdela O. Evaluation of warfarin anticoagulation at University of Gondar comprehensive specialized hospital, north-west Ethiopia. *J Blood Med.* 2021;12:189–195. doi:10.2147/JBM.S282948
34. Rosendaal FR, Cannegieter SC, Van der Meer FJM, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost.* 1993;69(3):236–239. doi:10.1055/s-0038-1651587
35. Thomson Micromedex. *Micromedex Healthcare Series [Intranet Database]. Version 5.1.* Greenwood Village, Colo: Thomson Micromedex; 2005.
36. Karuri WS. *Quality of Oral Anticoagulation Management Among Patients on Follow Up At Kenyatta National Hospital.* University of Nairobi; 2016.
37. Liyew Z. Evaluation of Anticoagulation Outcome among Patients Taking Warfarin: a Single-Center Experience, Northwest Ethiopia. *Adv Hematol.* 2021;2021:5489.
38. Fenta TG, Assefa T, Alemayehu B. Quality of anticoagulation management with warfarin among outpatients in a tertiary hospital in Addis Ababa, Ethiopia: a retrospective cross-sectional study. *BMC Health Serv Res.* 2017;17(1):1–7. doi:10.1186/s12913-017-2330-0
39. Cirus T, Cichocka-Radwan A, Lelonek M. Factors affecting the quality of anticoagulation with warfarin: experience of one cardiac centre. *Kardiochirurgia I Torakochirurgia Pol.* 2015;12(4):334–340. doi:10.5114/kitp.2015.56784
40. McAlister FA, Wiebe N, Hemmelgarn BR. Time in therapeutic range and stability over time for warfarin users in clinical practice: a retrospective cohort study using linked routinely collected health data in Alberta, Canada. *BMJ Open.* 2018;8(1):1–9. doi:10.1136/bmjopen-2017-016980
41. Yimer NS, Abiye AA, Hussien SU, Tadesse TA. Anticoagulation control, outcomes, and associated factors in patients with atrial fibrillation receiving warfarin at tertiary care hospital in Ethiopia. *Clin Appl Thromb.* 2021;2:27. doi:10.1177/10760296211049786
42. Health P, Setting C. The quality of anticoagulation therapy among warfarin-treated patients with atrial fibrillation in a primary health care setting. *Medicina.* 2019;55:1–11. doi:10.3390/medicina55010015
43. Lertsanguansinchai P, Huntrakul A, Rungpradubvong V. Factors predicting poor anticoagulant control on warfarin in a Thai population with non-valvular atrial fibrillation (NVAf): the ACACHE score. *Int J Arrhythmia.* 2021;22(1). doi:10.1186/s42444-021-00038-8
44. Sa MA. Quality of vitamin K antagonist anticoagulation in Spain: prevalence of poor control and associated factors. *Revista Española de Cardiología.* 2015;68(9):761–768.
45. Hakeem H. Evaluation of quality of warfarin therapy by assessing patient s time in therapeutic range at a tertiary care hospital in Pakistan. *JPMA.* 2018;68(9):1339–1344.
46. Mwita JC, Francis JM, Oyekunle AA, Gaenamang M, Goepamang M, Magafu MGMD. Quality of anticoagulation with warfarin at a tertiary hospital in Botswana. *Clin Applied Thromb.* 2017;1:596. doi:10.1177/1076029617747413
47. Task A, Members F, Hindricks G, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS) The Task Force for the diagnosis and management of atrial fibrillation of the Europe. *Clin Applied Thromb.* 2020;373–498.
48. McAuliffe GN, De Silva F, Upton A, Chan G. International normalised ratio monitoring in the community populations of the Auckland and Northland regions of New Zealand: time in therapeutic range and frequency of testing. *Int Med J.* 2018;48(12):1487–1491. doi:10.1111/imj.14032
49. de Barros e Silva PGM, Szejder H, Vasconcellos R, et al. Anticoagulation therapy in patients with non-valvular atrial fibrillation in a private setting in Brazil: a real-world study. *Arq Bras Cardiol.* 2020;114(3):457–466. doi:10.36660/abc.20180076
50. Teklay G, Shiferaw N, Legesse B, Bekele ML. Drug-drug interactions and risk of bleeding among inpatients on warfarin therapy: a prospective observational study. *Thrombosis J.* 2014;12(1):1–8. doi:10.1186/1477-9560-12-20

51. Tadesse TA, Woldu MA. Prevalence of warfarin drug interaction and warfarin education practice in outpatient setups of university teaching hospital. *J Basic Clin Pharm.* **2018**;262–266.
52. Liyew Z, Tadesse A, Bekele N, Tsegaye T. Evaluation of anticoagulation control among patients taking warfarin in University of Gondar Hospital, Northwest Ethiopia. *Adv Hematol.* **2021**;2021:1–8. doi:10.1155/2021/7530997
53. Feungfu L. Evaluation of time in therapeutic range among patients receiving warfarin therapy: a retrospective cohort study at one private hospital in Thailand. *Int J Med.* **2021**;48(2):107–114.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Identification of Laboratory Biomarkers for Early Detection and Clinical Management of Post-Acute Syndrome Among Survivors of the 2013–2016 West Africa Ebola Outbreak in Sierra Leone

Raoul Emeric Guetiya Wadoun, Stephen Sevalie, Maurice Baimba Kargbo, Andrew Clarke, Sherry Bangura, Mariatu Kargbo, Hawa Mariama Sesay, Abdul H Kamara, Jamil Bangura, Alie F Kamara, Sophie Allieu, Hassan Rogers, Maurizio Mattei, Vittorio Colizzi, Carla Montesano & Edwin JJ Momoh

To cite this article: Raoul Emeric Guetiya Wadoun, Stephen Sevalie, Maurice Baimba Kargbo, Andrew Clarke, Sherry Bangura, Mariatu Kargbo, Hawa Mariama Sesay, Abdul H Kamara, Jamil Bangura, Alie F Kamara, Sophie Allieu, Hassan Rogers, Maurizio Mattei, Vittorio Colizzi, Carla Montesano & Edwin JJ Momoh (2023) Identification of Laboratory Biomarkers for Early Detection and Clinical Management of Post-Acute Syndrome Among Survivors of the 2013–2016 West Africa Ebola Outbreak in Sierra Leone, *Journal of Blood Medicine*, , 119-132, DOI: [10.2147/JBM.S371239](https://doi.org/10.2147/JBM.S371239)

To link to this article: <https://doi.org/10.2147/JBM.S371239>



© 2023 Guetiya Wadoun et al.



Published online: 11 Feb 2023.



[Submit your article to this journal](#)



Article views: 291



[View related articles](#)



[View Crossmark data](#)



Citing articles: 1 [View citing articles](#)

Identification of Laboratory Biomarkers for Early Detection and Clinical Management of Post-Acute Syndrome Among Survivors of the 2013–2016 West Africa Ebola Outbreak in Sierra Leone

Raoul Emeric Guetiya Wadoun^{1,*}, Stephen Sevalie^{2,*}, Maurice Baimba Kargbo³, Andrew Clarke^{4,5}, Sherry Bangura⁶, Mariatu Kargbo⁶, Hawa Mariama Sesay⁷, Abdul H Kamara⁶, Jamil Bangura⁶, Alie F Kamara⁶, Sophie Allieu¹, Hassan Rogers¹, Maurizio Mattei⁸, Vittorio Colizzi⁹, Carla Montesano⁸, Edwin JJ Momoh^{1,3}

¹Department of Public Health, Microbiology and Immunology, Ernest Bai Koroma University of Science and Technology, Makeni, Sierra Leone; ²34th Military Hospital, The Republic of Sierra Leone Armed Forces, Freetown, Sierra Leone; ³Department of Agriculture and Food Security; Ernest Bai Koroma University of Science and Technology, Makeni, Sierra Leone; ⁴Global Programs Division, Save the Children UK, London, UK; ⁵Department/Division of Health Research, Lancaster University, Lancaster, UK; ⁶Sierra Leone Association of Ebola Survivors, Freetown, Sierra Leone; ⁷Department of Public Health, University of Makeni, Makeni, Sierra Leone; ⁸Department of Biology, University of Rome Tor Vergata, Rome, Italy; ⁹Department of Sciences and Technology, Evangelical University of Cameroon, Bandjoun, Cameroon

*These authors contributed equally to this work

Correspondence: Raoul Emeric Guetiya Wadoun; Edwin JJ Momoh, Email ergwadoun@ebkustsl.edu.sl; raoulemeric@gmail.com; emomoh@ebkust.edu.sl

Background: The clinical management of persistent medical conditions affecting Ebola survivors, generally described as a post-Ebola syndrome, remains a public health concern. We aimed to analyze Ebola survivors' laboratory biomarkers as compared to their non-infected household relatives to identify biomarkers that could guide the identification of survivors at increased risk of developing severe at odds with the non-severe post-Ebola syndrome.

Materials and Methods: Data were extracted from medical records of the Ebola survivors clinic, and we included only Ebola survivor's parameters recorded during the first baseline follow-up visit 2 weeks interval after their second negative PCR result. Moreover, household non-infected family contacts of survivors visiting the clinic during the same period were recruited as community control.

Results: The mean age of survivors was 32.65 (IQR: 15.5, 38.25) years, and Ebola IgG immunoglobulin was detected in all, thus confirming their status. The statistical significance (all $p < 0.05$) observed in monocyte percentage (MONO%), cluster of differentiation 4 percentage (CD4%), alanine aminotransferase (ALT), creatinine (CREA), and creatinine kinase (C-kinase) proved to be clinically significant as compared to the household relatives' group. Interestingly, the linear regression analysis indicated that the duration at ETU was negatively associated with lymphocyte percentage with a 5% lymphocyte decrease per day spent at ETU. Finally, there was a significant ($p < 0.05$) association between hematological (Hb, PCV, MCV, MCH), biochemical (ALT, CREA, C-kinase, T-cholesterol, triglycerides) parameters and the risk of developing severe complications.

Conclusion: We recommend clinicians closely monitor Hb, PCV, MCV, MCH, ALT, CREA, C-kinase, T-cholesterol, triglycerides and lymphocytes as clinically relevant laboratory biomarkers to identify survivors at higher risk of developing severe post-acute syndrome upon discharge from Ebola treatment unit including headache, abdominal pain, chest pain, ocular complication, arthralgia, hearing difficulty and erectile dysfunction which can impact health-related quality of life among Ebola survivors.

Keywords: Ebola virus disease, hematological, immunological, biochemical, biomarkers, post-acute syndrome

Introduction

The burden of the unprecedented West Africa Ebola epidemic was highest in Sierra Leone, with 14,124 reported cases and 3956 confirmed deaths.¹ Despite the deathly nature of the disease with an average case fatality ratio of 50%,

thousands survived and most often suffer from persistent clinical complications after hospital discharge described as post-Ebola sequelae.^{2,3} These complications often emanate from symptoms developed during acute infections with Ebola virus disease (EVD) exacerbated by the unavailable treatment for EVD. Clinical management during infection is mainly supportive, including administration of oral and intravenous fluids and electrolytes, co-infections, and symptom control. Moreover, EVD infection causes substantial biochemical, haematological and immunological abnormalities, which might be challenging to amend even after recovery.⁴ Therefore, the identification of laboratory predictors of the progression of complications towards severe or fatal form is urgently needed. These predictors will enable risk stratification, guide interventional follow-up to monitor survivors at enhanced risk of developing severe complications, and optimize limited technical and human resources allocation.

This study aimed to analyze Ebola survivors' laboratory biomarkers as compared to their non-infected household relatives to determine biomarkers that could guide the identification of survivors at increased risk of developing severe at odds with the non-severe post-Ebola syndrome.

Materials and Methods

Ethical Consideration

The study was approved by the Sierra Leone Ethics and Scientific Review Committee and the Sierra Leone Ministry of Health and Sanitation. All study participants or guardians approve to be enrolled by signing or fingerprinting a written consent form following the 1964 Helsinki declaration and later amendments.

Description of 34th Regimental Military Hospital Ebola Survivor Clinic

Ebola survivor clinics were established nationwide by the government with support from local and international partners to manage sequelae as soon as the number of patients recovering from EVD started increasing. The MH34 clinic was one of the first established and started enrolment of survivors and their family relatives 2 weeks (~14 days) after the discharge of the first cohort from the MH34 Ebola Treatment Unit (ETU). Inclusion criteria for EVD survivors were confirmed EVD by polymerase chain reaction testing and/or IgM/IgG positive on serological testing plus verification of EVD discharge certificate. At the same time, household contacts were expected to be close family non-infected relatives living together during the outbreak and have never been admitted to an ETU as well as not enrolled in an EVD vaccine clinical trial.⁴ Moreover, survivors were recruited regardless of ETU initially treated, and medical care was provided even if survivors were not willing to be enrolled in the study.

Furthermore, no direct compensation was provided to them other than the potential benefits of clinical examination and blood analysis. All medical care, laboratory diagnosis and treatment were provided for free. Personal history was recorded during each medical consultation, and a medical examination was performed, including psychological assessment and counselling.

Data Collection

All the records of laboratory-confirmed EVD survivors and their household members were extracted for a detailed review. Data available included socio-demographic and clinical characteristics, sequelae, haematological, biochemical, and immune biomarkers.

Laboratory Methods

Ebola Virus RNA Detection by qRT-PCR and Anti-EBOV IgG Quantification Using ELISA

All samples were initially examined to detect the Ebola virus ribonucleic acid (RNA) using a real-time quantitative polymerase chain reaction (qRT-PCR) assay to ascertain the absence of the virus after the onset of symptoms in survivors. Total RNA was extracted from plasma manually using a QiaAmp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's protocol, and materials were eluted in 60 µL of AVE buffer and stored at -80 °C until needed. The assay was performed on a LightCycler 96 thermocycler (Roche) using the RealStar Filovirus Screen RT-PCR 1.0 kit from Altona Diagnostics and considered positive if cycle threshold (Ct) values were <40.

All qRT-PCR negative samples were subjected to human anti-glycoprotein (GP) IgG responses to *Zaire Ebola virus* using an enzyme-linked immunosorbent assay (ELISA) commercial kit from Alpha Diagnostic International (ADI, Texas, USA) according to the manufacturer's instructions. Positive control and calibrators provided with the kit were used in each test run, and optical density was read at 450 nm on a MultiScanGo microplate reader (ThermoFisher Scientific) with plasma diluted at 1:200. Antibody detection was further validated in oral fluid samples from identical participants by the use of a new IgG capture assay based on the EBOV Mayinga GP antigen (rGPδTM [catalogue 0501-016]; IBT Bioservices, Rockville, MD, USA) as described by Lambe et al⁵ and the presence of antibody was used as additional evidence for survivor status.

Clinical Laboratory Assays

Anticoagulated whole blood was used for assessment of complete blood count with a five-part differential automated haematology analyzer (Beckman Coulter, CA, USA), including haemoglobin (Hb), packed cell volume/hematocrit (PCV/HCT), leukocyte (WBC), mean corpuscular volume (MCV), red blood cell count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), basophile (BASO), eosinophils (EOSIN), neutrophils (NEUT), monocytes (MONO), and lymphocytes (LYM).

Another blood portion was used for a cluster of differentiation 4 (CD4) cell count and CD4 percentage analysis with a FACSCalibur flow cytometer (Becton Dickinson Immunochemistry, California, USA). Serum chemistries were performed on cryopreserved samples on a Roche Reflotron Plus clinical chemistry analyzer with manufacturer reagent strips specific to each of the following parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin (BIL), creatinine (CREA), urea, creatine kinase (C-kinase), pancreatic amylase (P-amylase), total cholesterol (T-cholesterol), and triglycerides.

All biomarkers were compared with clinical reference values established by the World Health Organization to identify clinical differences.⁶

Statistical Analysis

Data were transformed into a Microsoft Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) Version 20.0. Descriptive statistics, mean, standard deviations and interquartile range (IQR) were used to present the findings. The analysis of biomarkers levels in Ebola survivors against household relatives was performed using Mann–Whitney *U*-tests. Multivariate correlation for biomarkers level and survivor duration at the ETU were analysed with the nonparametric Spearman's test (alpha 0.05) running on a JMP Software, v13.1.0 (SAS Institute, Cary, NC, USA). A colour map matrix represented correlations (Spearman *r*). A point-biserial correlation was further performed to ascertain whether the post-Ebola syndrome was associated with various biomarkers. A linear regression model was used to establish whether the biomarkers significantly higher in survivors than relatives were predicted by the duration at the ETU.

Results

A total of 213 participants were enrolled in the present study, including 83 EVD survivors and 133 household relatives of survivors (Figure 1). Their characteristics are outlined in Table 1.

The mean age of survivors was 32.65 years (IQR: 15.5, 38.25). In addition to the clinical report and discharge certificate review, EVD survivor status was confirmed using combined serological assays to detect Ebola virus IgG response (Figure 2).

Both serological methods proved effective in distinguishing survivors from their household non-infected relatives, as evidenced by the positive correlation ($r = 0.476$) between the IgG antibody activity in the serum and saliva of survivors (Figure 3).

The analysis of haematological biomarkers showed that haemoglobin concentration and red blood cell count were significantly different in the survivor group than in household relatives. A similar pattern was observed with the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration ($p < 0.05$), as shown in Table 2. However, there is no significant difference in white blood cells, haematocrit and platelet count between

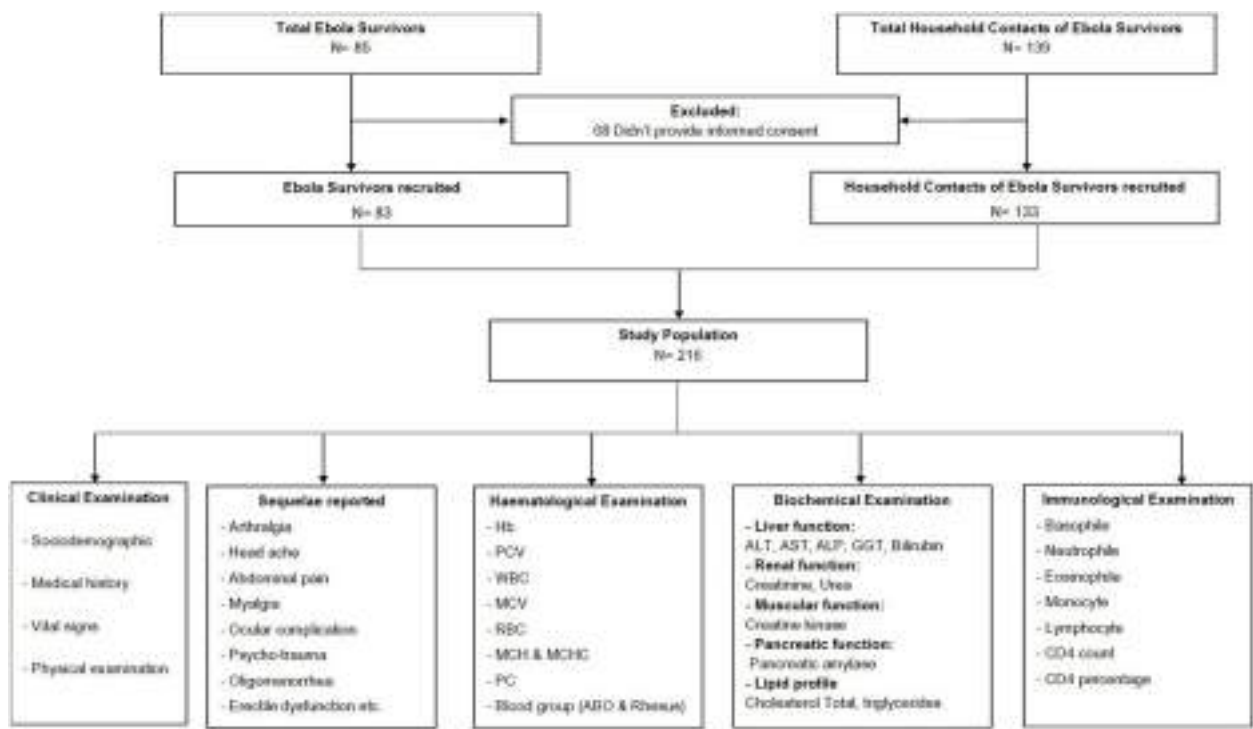


Figure 1 Study flow chart.
Abbreviations: Hb, haemoglobin; PCV/HCT, packed cell volume/haematocrit; WBC, leukocyte's concentration; RBC, red blood cell count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; CD4, cluster of differentiation 4; CD4, cluster of differentiation percentage; ALT, alanine amino-transferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

the survivors and household relative's cohort (all $p > 0.05$). Regarding haemoglobin and mean corpuscular volume estimation, the survivors' cohort significantly reported a higher level for both than the household relative's cohort ($p < 0.05$).

As displayed in Table 2, the immunological profile of biomarkers shows that the monocyte count and CD4 percentage were significantly higher in the survivor's group than in the household relatives' group with $p < 0.001$ and 0.04 , respectively. Nevertheless, there was no significant difference in basophil, eosinophil, neutrophil, lymphocyte and CD4 count in both cohorts ($p > 0.05$).

Analysis of the biochemical profile showed that the proportion of these biomarkers significantly higher was more relevant in the survivor cohort than the household relative cohort. The findings indicated that the survivor cohort had

Table 1 Demographic and Clinical Characteristics of Survivors and Their Household Relatives

Characteristic	Mean $\pm$ SD, Frequency/Total (%)	
	Ebola Survivors, N= 83	Household Relatives, N=133
Serological profile and Ebola virus detection		
Ebola virus RNA	Not detected	Not detected
IgG – Ebola virus glycoprotein		
Seropositive	83/83 (100)	06/133 (04.5)
Seronegative	00/83 (100)	127/133 (95.49)
Age (mean SD), years	32.65 $\pm$ 8.85	27.60 $\pm$ 11.28
<40	68/83 (81.92)	116/133 (87.22)
≥ 40	15/83 (18.07)	17/133 (12.78)

(Continued)

Table I (Continued).

Characteristic	Mean $\pm$ SD, Frequency/Total (%)	
	Ebola Survivors, N= 83	Household Relatives, N=133
Gender		
Male	34/83 (40.96)	80/133 (60.15)
Female	48/83 (57.83)	53/133 (39.85)
Not recorded	01/83 (1.20)	00/133 (00.00)
Marital status		
Single	32/83 (38.55)	86/133 (64.66)
Married	51/83 (61.44)	47/133 (35.34)
Clinical parameters		
Temperature	36.74 $\pm$ 0.53	36.48 $\pm$ 0.26
Respiratory rate, (/min)	18.58 $\pm$ 1.59	17.71 $\pm$ 2.84
Heart rate, b/min	73.90 $\pm$ 10.91	73.21 $\pm$ 12.96
Systolic blood pressure (mmHg)	112.69 $\pm$ 14.44	114.39 $\pm$ 16.52
Diastolic blood pressure (mmHg)	66.92 $\pm$ 11.61	67.00 $\pm$ 12.80
Height (cm)	166.10 $\pm$ 8.52	169.02 $\pm$ 9.12
Weight (kg)	65.69 $\pm$ 10.98	65.88 $\pm$ 14.04
A+	14/83 (16.86)	28/133 (21.05)
A-	01/83 (01.20)	00/133 (00.00)
AB+	03/83 (03.61)	06/133 (04.51)
B+	25/83 (30.12)	27/133 (20.30)
B-	00/83 (00.00)	02/133 (01.50)
O+	38/83 (45.78)	67/133 (50.38)
O-	02/83 (02.41)	01/133 (0.75)
Comorbidities/Clinical history prior to EVD		
Chronic cardiac disease	00/83	00/133
Cancer	00/83	00/133
Chronic pulmonary disease	00/83	00/133
Asthma physician-diagnosed	00/83	00/133
Renal disease	00/83	00/133
Obesity	00/83	00/133
Diabetes	00/83	00/133
Rheumatologic disease	00/83	00/133
Chronic neurological dementia	00/83	00/133
Hemiplegia/paraplegia	00/83	00/133
HIV/AIDS	00/83	00/133
Tuberculosis	00/83	00/133
Lassa fever disease	00/83	00/133
Post-Ebola syndrome/sequelae after ETU		
Arthralgia	62/83 (74.69)	11/133 (08.27)
Hearing difficulty/ringing sounds	01/83 (01.20)	01/133 (0.75)
Headache	56/83 (67.47)	05/133 (03.76)
Abdominal pain	08/83 (09.63)	04/133 (03.00)
Chest pain	12/83 (14.46)	00/133 (00.00)
Diarrhoea	01/83 (01.20)	00/133 (00.00)
Myalgia	62/83 (74.69)	02/133 (01.50)
Ocular complication	10/83 (12.04)	00/133 (00.00)
Constipation	02/83 (02.40)	00/133 (00.00)
Insomnia	03/83 (03.61)	00/133 (00.00)
Oligomenorrhea (Female)	00/83 (00.00)	00/133 (00.00)
Erectile dysfunction (male)	02/83 (02.40)	00/133 (00.00)

Abbreviation: ETU, Ebola Treatment Unit.

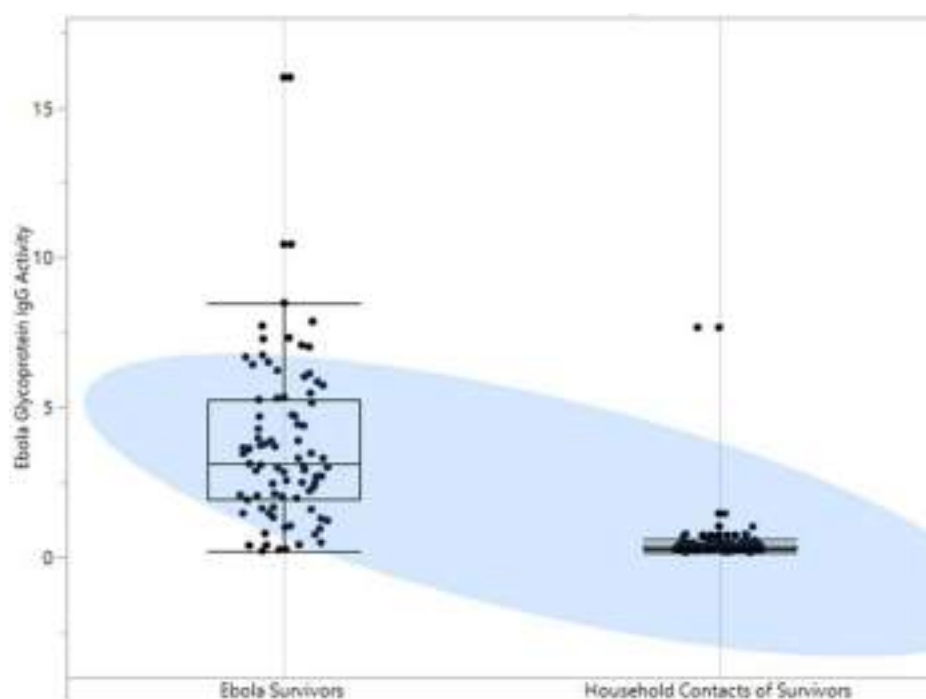


Figure 2 Ebola IgG antibody level in survivors and their household non-infected relatives.

Abbreviation: IgG, immunoglobulin G.

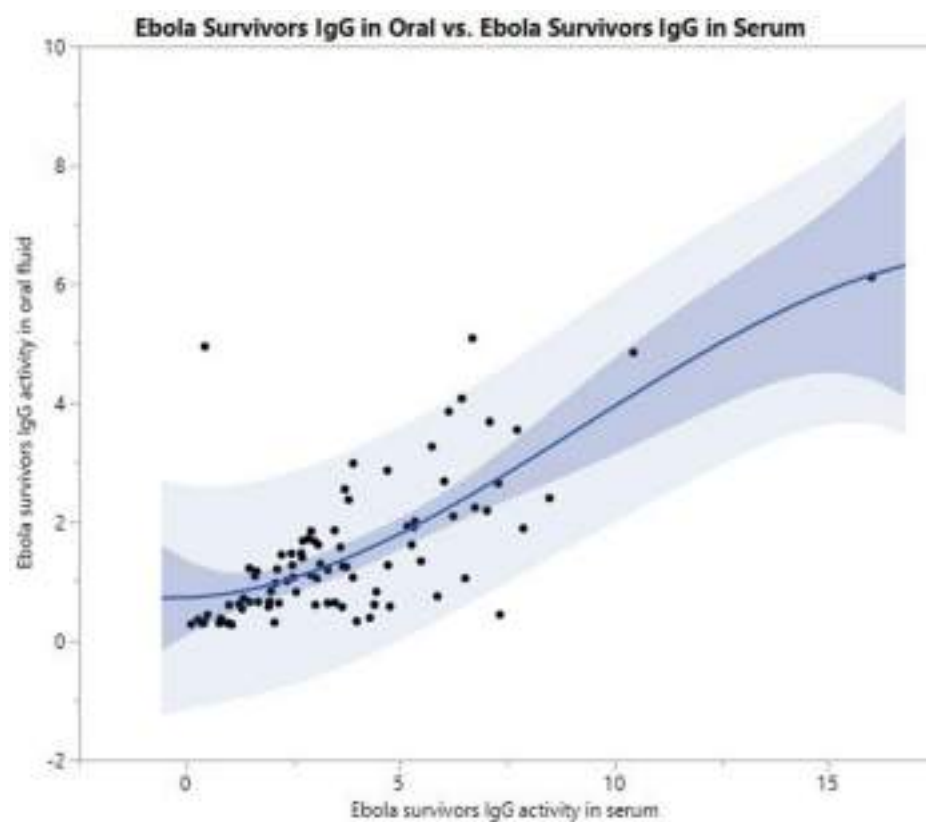


Figure 3 Quadratic correlation representation between Ebola IgG antibody level in serum and oral fluid of survivors.

Abbreviation: IgG, immunoglobulin G.

Table 2 Comparison of Clinical Biomarkers of Survivors and Their Household Relatives

Biomarkers	Mean ± SD		P-value*
	Survivors (n=83)	Household Relatives (n=133)	
Haematology			
Hb, g/L	12.47±1.37	11.93±1.13	0.040*
PCV/HCT, %	37.49±4.11	38.27±27.45	0.060
WBC, ×10 ⁹ /L	5.40±1.21	5.67±1.19	0.306
MCV, fl	82.96±6.93	79.69±8.45	<0.001*
RBC, ×10 ⁶ /μL	4.68±0.58	4.88±0.61	0.017*
MCH, pg	29.13±2.05	29.95±1.53	0.001*
MCHC, g/dL	31.90±2.33	31.62±15.64	<0.001*
PLT, ×10 ⁹ / μL	231.90±56.65	212.27±43.61	0.052
Immunological			
BASO, %	0.31±0.32	0.29±0.41	0.070
EOSIN, %	3.43±0.53	3.64±5.1	0.806
NEUT, %	55.51±47.76	51.68±4.28	0.124
MONO, %	6.62±4.40	3.96±3.34	<0.001**
LYM, %	41.73±7.78	41.49±4.80	0.439*
CD4 count, cells/μL	1271.10±469.98	1241.14±500.36	0.709
CD4 percentage, %	40.20±10.22	37.79±11.97	0.045**
Biochemical			
ALT, U/L	62.35±14.45	28.45±16.83	<0.001**
AST, U/L	29.59±14.63	26.73±9.93	0.045*
ALP, U/L	104.14±56.47	113.61±68.10	0.537
GGT, U/L	22.06±5.31	25.21±3.89	<0.001*
BIL, μmol/L	14.67±6.44	12.82±5.27	0.049*
CREA, μmol/L	121.97±69.68	75.92±20.469	<0.001**
UREA, μmol/L	4.92±1.68	4.91±1.41	0.744
C-kinase, U/L	216.22±92.19	112.10±127.82	<0.001**
P-amylase, U/L	57.11±19.22	59.30±20.197	0.459
T-cholesterol, mg/dL	146.14±56.47	182.33±68.94	<0.001*
Triglycerides, mg/dL	118.94±65.97	127.11±65.97	0.308

Notes: *P<0.05 was considered statistically significant. **Clinically significant difference.

Abbreviations: Hb, haemoglobin; PCV/HCT, packed cell volume/haematocrit; WBC, leukocyte's concentration; MCV, mean corpuscular volume; RBC, red blood cell count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; BASO, basophile; EOSIN, eosinophils; NEUT, neutrophils; MONO, monocytes; LYM, lymphocytes; CD4, cluster of differentiation 4; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BIL, bilirubin; CREA, creatinine; C-kinase, creatine kinase; P-amylase, pancreatic amylase; T-cholesterol, total cholesterol.

a significantly higher ($p < 0.05$) concentration of alanine transaminase, aspartate transaminase, bilirubin, creatinine and creatine kinase than the household relative cohort. Besides, the level of alkaline phosphatase, urea, and triglyceride was not significantly different (all $p > 0.05$) in both cohorts. Nonetheless, gamma-glutamyl transferase and total cholesterol levels were significantly higher in the household relative cohort than in the survivor cohort ($p < 0.05$).

Moreover, a Spearman correlation was conducted to examine the relationship between haematological, immunological and biochemical biomarkers with the Ebola survivor duration at the ETU (Table 3).

Overall, weak non-significant correlations were observed for several biomarkers and the duration at ETU. However, we observed a weak positive significant correlation between the duration at ETU and the mean corpuscular haemoglobin (Spearman $r = 0.23$; $N=83$; $p = 0.037$) as well as with alkaline phosphatase (Spearman $r = 0.25$; $N=83$; $p = 0.023$); while a weak negative significant correlation was observed between the duration at ETU and lymphocyte (Spearman $r = -0.24$;

Table 3 Biomarkers' Correlation with Survivor Duration at ETU and Presence or Absence of Some Post-Acute Sequelae

Biomarkers	Duration at ETU	Post-Acute Sequelae											
		Arthralgia	Hearing Difficulty	Headache	Abdominal Pain	Chest Pain	Diarrhea	Myalgia	Ocular Complication	Constipation	Insomnia	Oligomenorrhea	Erectile Dysfunction
	Spearman <i>r</i> / P-value	<i>r</i> _{pb} /p-value											
Haematological													
Haemoglobin	− 0.009/0.93	0.118/0.28	0.121/0.27	0.123/0.26	0.82/0.46	−0.082/0.46	−0.124/0.26	−0.093/0.26	−0.252/0.02*	−0.068/0.58	−0.122/0.27	—***	−0.119/0.29
PCV/ hematocrit	− 0.028/0.80	0.086/0.44	0.93/0.40	0.11/0.32	0.077/0.49	−0.095/0.39	−0.122/0.27	−0.108/0.33	−0.263/0.016*	−0.77/0.49	−0.12/0.28	—	−0.12/0.30
White blood cell	0.112/0.31	0.023/0.83	−0.075/0.50	−0.023/ 0.84	−0.031/0.78	−0.076/0.49	0.112/0.31	0.079/0.48	−0.00/0.97	0.066/0.56	0.00/0.33	—	0.00/0.81
MCV	0.00/0.94	0.156/0.16	0.03/0.79	0.22/0.04*	0.161/0.15	0.043/0.70	0.062/0.58	−0.021/0.85	−0.050/0.66	0.020/0.86	0.109/0.33	—	0.077/0.49
RBC	0.018/0.87	0.087/0.44	0.114/0.30	0.032/0.77	−0.052/0.64	−0.12/0.28	−0.061/0.58	0.030/0.79	−0.147/0.19	0.162/0.14	−0.1108/0.33	—	0.038/0.74
MCH	0.03/0.78	0.129/0.24	0.00/0.97	0.131/0.24	0.255/0.02*	0.22/0.04*	0.169/0.12	0.118/0.29	0.112/0.27	0.083/0.46	0.199/0.071	—	0.201/0.74
MCHC	0.016/0.88	0.72/0.52	0.087/0.43	0.1/0.37	−0.114/0.30	−0.136/0.22	0.177/0.11	−0.56/0.61	−0.045/0.68	−0.046/0.67	−0.183/0.09	—	−0.012/0.91
Platelets	− 0.00/0.95	−0.106/0.34	0.027/0.80	−0.152/ 0.17	−0.055/0.62	0.019/0.86	−0.065/0.56	−0.031/0.79	0.176/0.11	−0.166/0.13	−0.178/0.107	—	−0.057/0.61
Immunological													
Basophil	0.115/0.298	0.059/0.59	0.106/0.34	0.171/0.12	−0.105/0.34	0.117/0.29	−0.031/0.78	−0.083/0.455	−0.071/0.52	−0.02/0.855	−0.035/0.75	—***	0.028/0.79
Eosinophil	0.00/0.95	0.18/0.10	0.045/0.68	0.117/0.29	−0.039/0.73	−0.18/0.10	0.076/0.51	−0.033/0.76	−0.015/0.89	−0.109/0.33	−0.116/0.29	—	0.131/0.24
Neutrophil	− 0.00/0.93	−0.134/ 0.226	0.00/0.96	−0.123/ 0.27	0.054/0.63	0.073/0.51	0.00/0.94	−0.124/0.26	0.076/0.51	0.00/0.97	0.026/0.81	—	0.023/0.84
Monocyte	− 0.061/0.58	0.101/0.34	0.115/0.29	−0.013/ 0.90	−0.068/0.54	−0.132/0.23	0.015/0.89	0.077/0.49	−0.043/0.69	−0.051/0.65	−0.092/0.41	—	0.128/0.25
Lymphocyte	− 0.224/0.041*	0.087/0.43	−0.018/0.87	−0.024/ 0.83	0.020/0.86	0.091/0.41	0.025/0.83	0.157/0.16	−0.075/0.49	0.116/0.29	0.035/0.75	—	−0.036/0.75
CD4 count	0.105/0.34	−0.146/0.19	−0.12/0.28	−0.178/ 0.11	−0.027/0.81	−0.00/0.99	0.087/0.44	−0.169/0.13	0.113/0.31	0.087/0.44	−0.00/0.97	—	0.123/0.75
CD4 percentage	− 0.053/0.63	−0.081/0.47	−0.138/0.22	−0.120/ 0.28	−0.176/0.12	−0.067/0.55	0.068/0.55	−0.00/0.97	−0.053/0.64	0.68/0.55	−0.052/0.64	—	0.127/0.26

Biochemical													
ALT	-0.085/0.44	-0.240/ 0.029*	-0.181/0.10	-0.227/ 0.039*	-0.029/0.80	0.096/0.39	0.056/0.61	-0.144/0.19	0.145/0.19	0.015/0.89	-0.018/0.87	—***	0.042/0.71
AST	0.049/0.65	-0.036/ 0.749	0.034/0.76	-0.101/ 0.36	0.067/0.55	0.127/0.25	0.37/0.74	-0.056/0.62	0.145/0.19	0.040/0.72	0.017/0.88	—	0.056/0.62
ALP	0.146/0.18	0.036/0.75	-0.232/0.03	0.00/0.96	0.114/0.31	0.043/0.69	0.12/0.28	-0.036/0.75	0.09/0.42	0.166/0.14	0.193/0.08	—	0.094/0.39
GGT	-0.029/0.79	-0.00/0.97	-0.062/0.58	-0.010/ 0.93	-0.013/0.90	-0.056/0.62	-0.062/0.59	-0.00/0.97	0.17/0.88	-0.088/0.43	0.05/0.65	—	0.031/0.78
BIL	-0.066/0.55	-0.129/0.25	0.115/0.30	0.011/0.92	0.21/0.053	0.140/0.20	-0.023/0.84	-0.171/0.12	0.068/0.54	0.029/0.79	0.00/0.99	—	-0.020/0.86
CREA	-0.013/0.2	0.049/0.66	0.065/0.56	0.226/ 0.04*	0.100/0.37	0.116/0.29	0.041/0.71	-0.029/0.79	0.107/0.33	0.00/0.99	-0.016/0.89	—	-0.487/ 0.00**
Urea	-0.191/0.08	-0.044/0.69	-0.020/0.86	0.025/0.82	0.083/0.45	0.063/0.59	-0.00/0.99	0.09/0.37	0.53/0.64	-0.033/0.76	0.054/0.63	—	0.01/0.9
C-kinase	0.044/0.68	0.109/0.33	0.149/0.18	0.099/0.37	0.169/0.13	0.216/0.04*	0.069/0.05	0.053/0.63	0.174/0.12	0.164/0.14	0.061/0.58	—	0.098/0.38
P-amylase	0.00/0.69	-0.056/0.61	0.120/0.28	-0.088/ 0.43	0.065/0.56	-0.056/0.62	0.095/0.39	-0.97/0.38	-0.058/0.60	0.085/0.45	0.104/0.35	—	0.107/0.34
T-cholesterol	0.022/0.99	0.131/0.24	-0.232/ 0.03*	0.065/0.56	-0.038/0.074	-0.060/0.59	0.183/0.09	0.00/0.95	-0.06/0.59	0.202/0.067	0.133/0.23	—	0.21/0.06
TG	-0.044/0.69	0.256/ 0.02*	0.01/0.93	0.178/0.11	0.041/0.71	-0.031/0.78	-0.101/0.36	0.057/0.36	0.031/0.78	-0.023/0.84	-0.148/0.18	—	-0.058/0.6

Notes: *Correlation significant at the 0.05 level (2-tailed); **Correlation significant at the 0.01 level (2-tailed); ***Could not be computed as at least one of the variables is constant; bold font indicates statistical significance.

$N=83$; $p=0.029$) same as with the platelet level (Spearman $r = -0.29$; $N=83$; $p=0.008$). All results were represented in a colour scatterplot matrix, where statistically supported associations ($p<0.05$) between duration at ETU and various biomarkers were highlighted (Figures 4–6).

Interestingly, there was a significant ($p < 0.05$) association between haematological (Hb, PCV, MCV, MCH), biochemical (ALT, CREA, C-kinase, T-cholesterol, triglycerides), and immune (lymphocytes%) parameters and the risk of developing severe complications (Table 3).

A simple linear regression model was calculated to predict the impact of the admission period at the Ebola Treatment Unit on survivors' haematological, immunological and biochemical biomarkers taken individually. The overall regression analysis demonstrated no significant prediction between the duration at the ETU and various biomarkers of survivors. However, a significant negative regression model was found between the duration at ETU and the lymphocyte percentage of survivors ($F(1, 81) = 4.29$, $p=0.0415$), with an R^2 of 0.05 (Figure 7).

Considering the lymphocyte percentage as y and the duration at ETU as x , the regression equation will be equal to $y = a + bx$; thus,

$$\text{Lymphocyte\%} = 43.28 - 0.05 * \text{Duration at ETU}$$

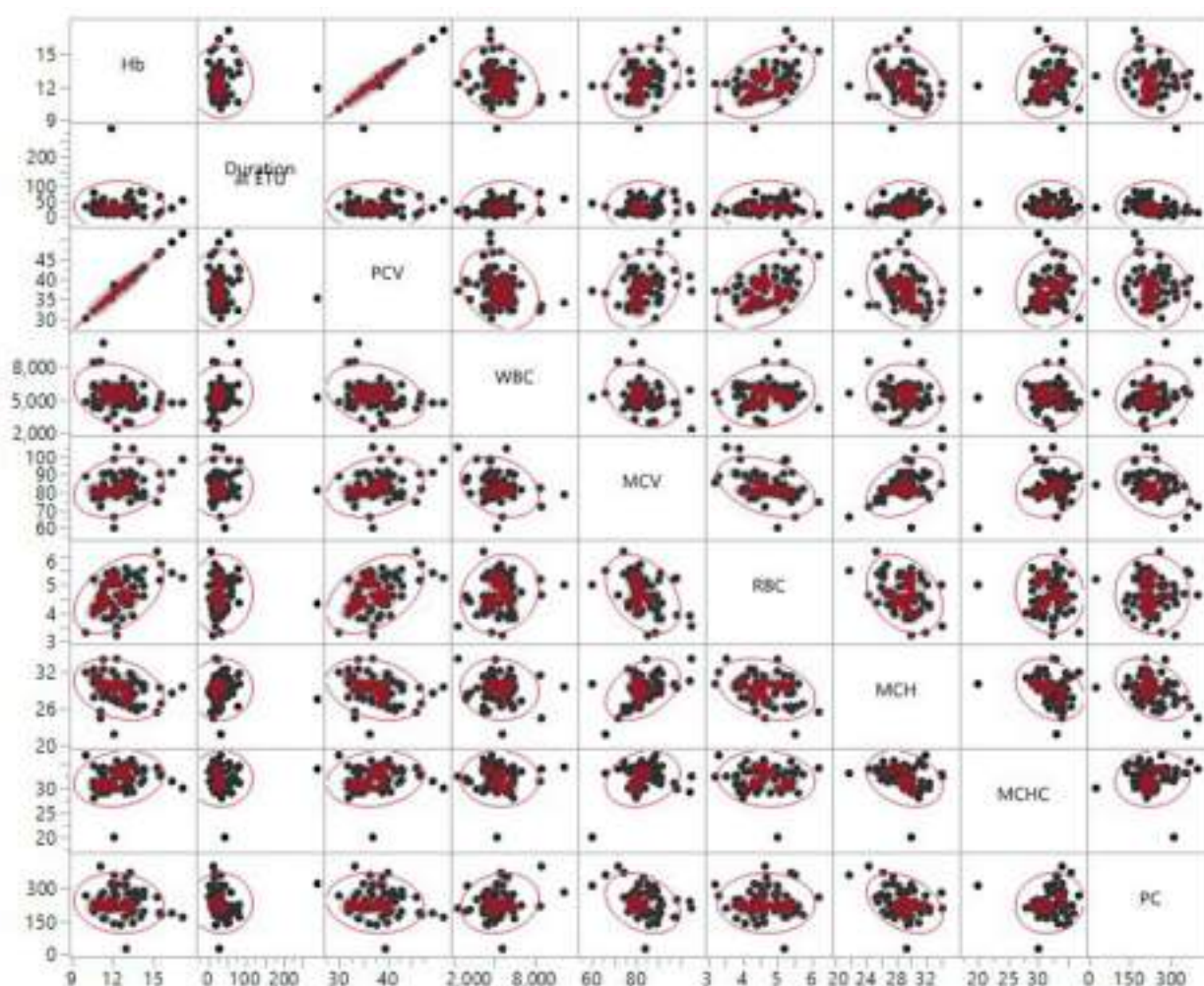


Figure 4 Non-parametric correlation between the duration at the ETU and haematological biomarkers of survivors.

Abbreviations: ETU, Ebola Treatment Unit; Hb, haemoglobin; PCV/HCT, packed cell volume/haematocrit; PC, platelets; WBC, leukocyte's concentration; RBC, red blood cell count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume.

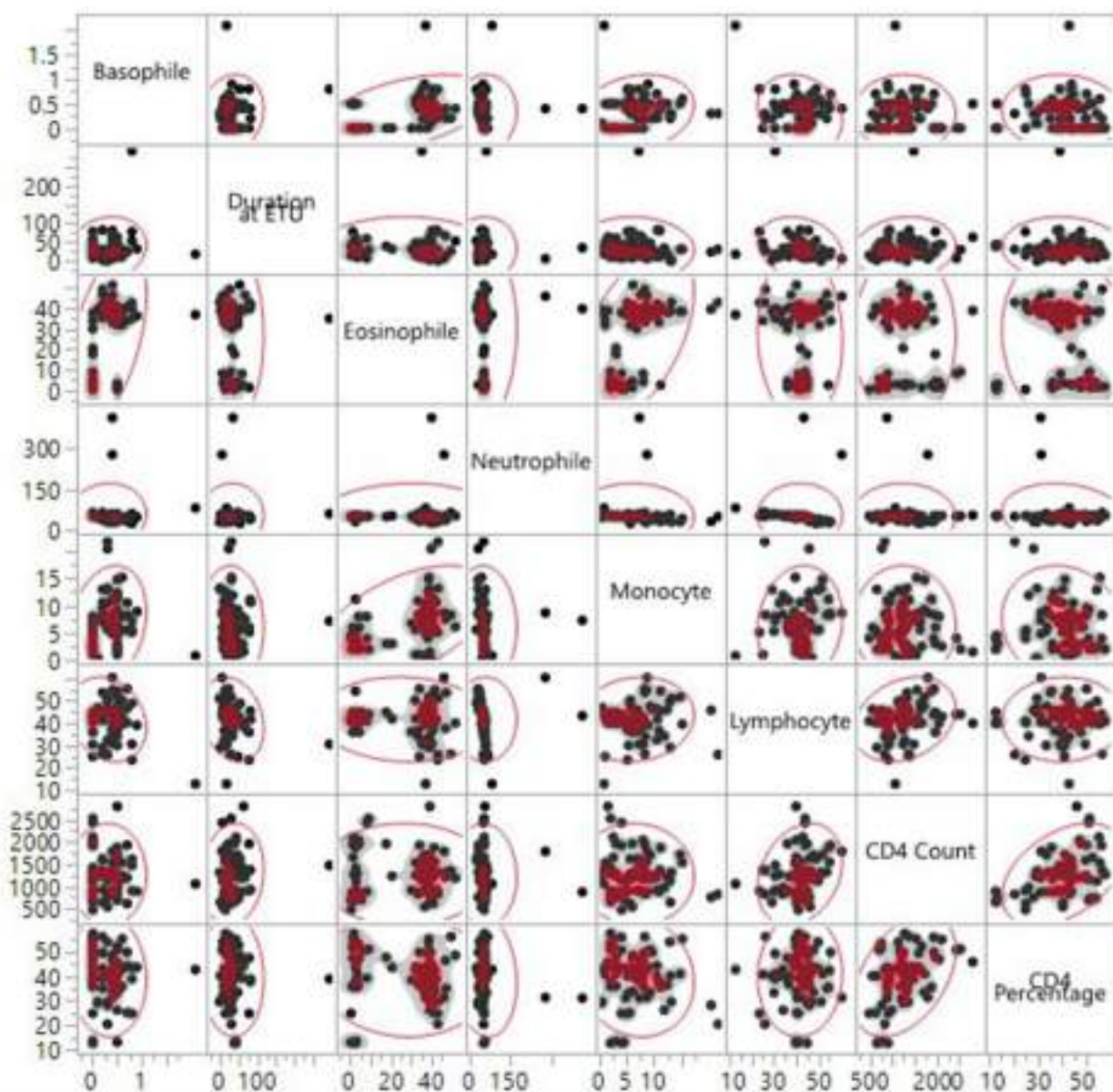


Figure 5 Non-parametric correlation between the duration at the ETU and immunological biomarkers of survivors.

Abbreviations: ETU, Ebola Treatment Unit; CD4, cluster of differentiation 4; CD4, cluster of differentiation percentage.

Discussion

With continuous medical complications reported by survivors, essential clinical care should be provided continuously following the WHO reference document on clinical care for survivors of EVD.⁴ However, good clinical decision-making requires appropriate medical laboratory investigations that are often expensive in sub-Saharan Africa, which hosts more than 90% of EVD survivors. Therefore, identifying key laboratory biomarkers with a clinical signature could rapidly guide risk stratification and monitoring of survivors at an enhanced level of developing severe complications. Unfortunately, little is known regarding the long-term effects of EVD on the haematological, immunological, and biochemical profile of patients after discharge from ETU, even though some preliminary studies provided relevant insights during acute infection.⁷

The study showed that post-Ebola syndrome remains a severe health concern affecting survivors with the most reported sequelae being arthralgia, myalgia, and persistent headache. These findings concur with previous studies reporting clinical sequelae of survivors after release from ETU.^{3,8}

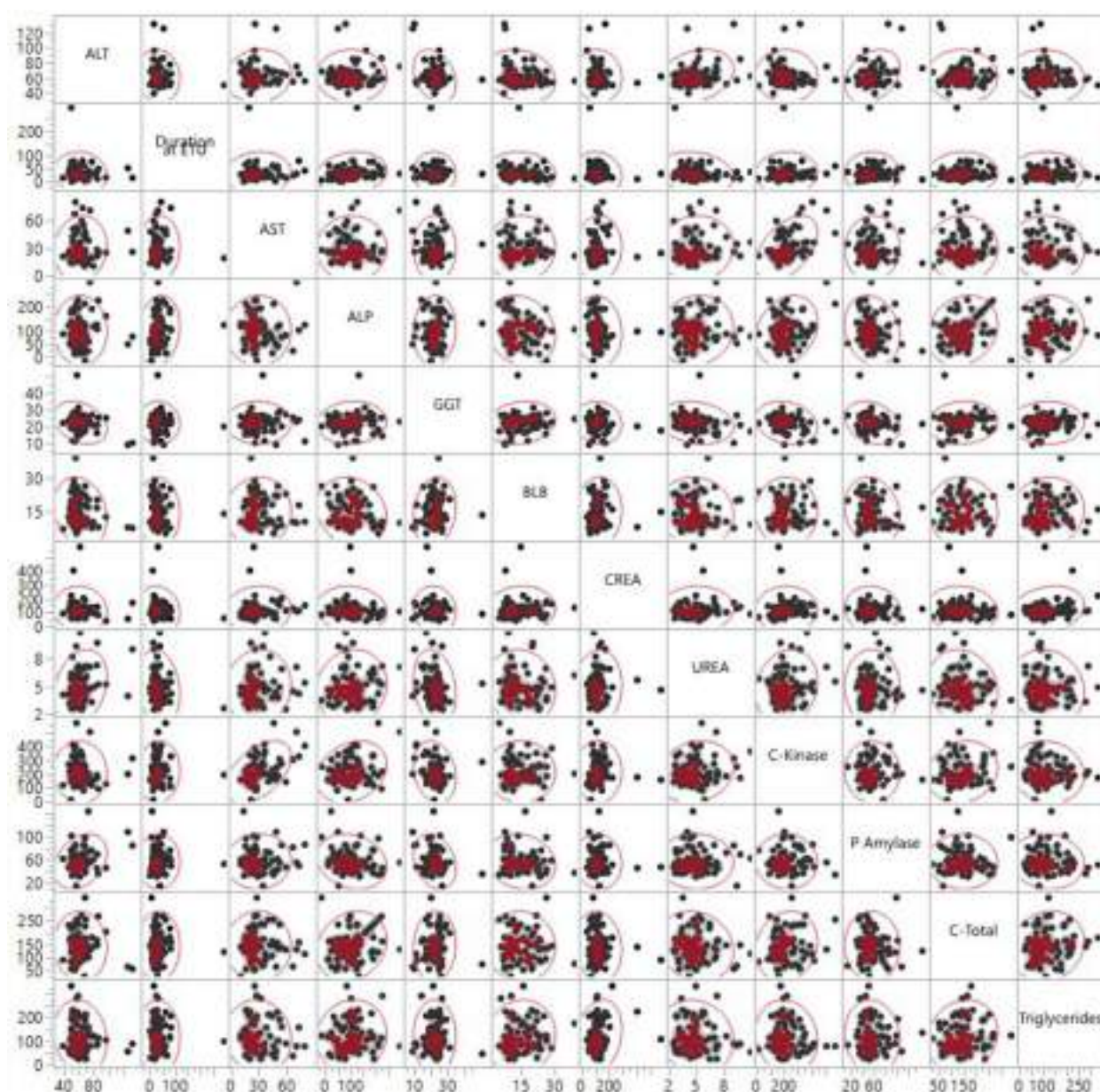


Figure 6 Non-parametric correlation between the duration at the ETU and biochemical biomarkers of survivors.

Abbreviations: ETU, Ebola Treatment Unit; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BLB, bilirubin; CREA, creatinine; C-Kinase, creatine kinase; P-Amylase, pancreatic amylase; C-total, total cholesterol.

A comparison of haematological biomarkers showed that the survivor cohort had higher Hb, MCV and platelet levels, but mostly still within normal ranges with a positive correlation observed between the duration at ETU and the mean corpuscular haemoglobin level. In contrast, this correlation was negative with platelet count. These findings align with related studies on viral haemorrhagic fever diseases.⁹

We also observed a significant difference among monocytes and CD4 percentage between survivors and their household relatives, similar to earlier studies.¹⁰ Even though there was no difference in lymphocytes between both cohorts, regression analysis indicated that duration at ETU during acute infection was negatively associated with lymphocytes percentage, with the equation indicating 5% lymphocytes decrease per day spent at ETU. This conclusion agrees with studies indicating that EVD is associated with pronounced lymphopenia that highly correlates with fatalities.¹⁰

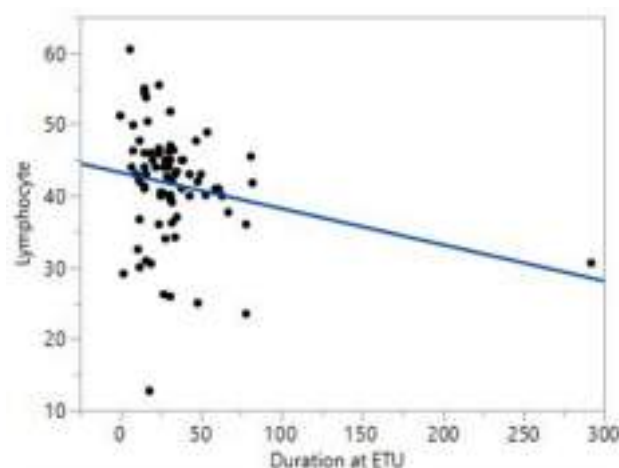


Figure 7 Linear regression model of the duration at the ETU and lymphocytes % of survivors.
Abbreviation: ETU, Ebola Treatment Unit.

In the present study, many biochemical biomarkers showed a significant difference between survivors and their household relatives, including AST, ALT, ALP, BIL, CREA, C-kinase, and triglycerides, with a correlation observed between duration at ETU and ALP. These abnormalities in these biomarkers suggested that EVD may damage hepatic, myocardial, renal, and other survivor's organs. These findings are consistent with earlier research describing complications associated with EVD.⁷ However, Clark et al did not observe any clinically relevant abnormalities in biochemistry biomarkers among 70 EVD survivors 3 years after discharge from ETU.¹¹

Generally, there were statistically significant differences between the two groups for several biomarkers. However, that is not always the same as a clinically significant difference. Some biomarkers in both groups were still within normal ranges; therefore, individual biomarkers alone might be difficult to predict for clinicians. However, describing a more concrete group of biomarkers from our findings will help typify and identify those most at risk.

Finally, the impact of the haematological, immunological, and biochemical variations described is still unclear regarding the cause and consequence of the persistence of sequelae and the vulnerability of survivors to other communicable and non-communicable diseases. In addition, the variations observed may be due to confounding factors such as co-infection with other infectious diseases and underlying unreported medical status. Therefore, further research is needed on a significant survivor population across multiple sites to reach a specific conclusion.

Limitations

This retrospective cross-sectional cohort study is limited to one EVD survivor's clinic and does not provide cause-and-effect relationships. These studied biomarkers require further investigation. By studying many survivors, we will surely gain more precise information about the long-term effects of the Ebola virus infection on haematological, immunological, and biochemical parameters in survivors and their potential clinical applications, which will further help improve their care.

Conclusion

This study provides new insights into the vital role of hematologic, immunological and biochemical laboratory biomarkers in managing clinical features in survivors of Ebola virus disease. We recommend clinicians closely monitor Hb, PCV, MCV, MCH, ALT, CREA, C-kinase, T-cholesterol, triglycerides and lymphocytes as clinically relevant biomarkers to identify survivors at higher risk of developing severe post-acute syndrome upon discharge from Ebola treatment unit including headache, abdominal pain, chest pain, ocular complication, arthralgia, hearing difficulty and erectile dysfunction, which can impact health-related quality of life among Ebola survivors. However, these biomarkers need further evaluation in a more significant number of survivors.

Data Sharing Statement

The authors declare that all data supporting the findings of this study are available within the article. The datasets generated, used, and analyzed during this study are available from the corresponding author Dr Raoul Emeric Guetiya Wadoun upon reasonable request.

Acknowledgments

We want to extend our thanks and appreciation to the Ebola survivors and their household relatives for their participation in this study. We also want to thank the Ministry of Health and Sanitation, the Ministry of Social Welfare, Gender and Children's Affairs, and the Sierra Leone Association of Ebola Survivors for their support.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

References

1. World Health Organization (WHO). Ebola data and statistics: situation summary data; 2016. Available from: <http://apps.who.int/gho/data/view Ebola-sitrep Ebola-summary-20160511?lang=en>. Accessed February 27, 2022.
2. Tiffany A, Vetter P, Mattia J, et al. Ebola virus disease complications as experienced by survivors in Sierra Leone. *Clin Infect Dis*. 2016;62(11):1360–1366. doi:10.1093/cid/ciw158
3. Guetiya Wadoun RE, Samin A, Mafopa NG, et al. Mobile health clinic for the medical management of clinical sequelae experienced by survivors of the 2013–2016 Ebola virus disease outbreak in Sierra Leone, West Africa. *Eur J Clin Microbiol Infect Dis*. 2017;36(11):2193–2200. PMID: 28695354. doi:10.1007/s10096-017-3045-1
4. WHO. Clinical care for survivors of Ebola virus disease: interim guidance. Available from: http://apps.who.int/iris/bitstream/10665/204235/1/WHO_EVD_OHE_PED_16.1_eng.pdf?ua=1. Accessed February 26, 2022.
5. Lambe T, Rampling T, Samuel D, et al. Detection of vaccine-induced antibodies to Ebola virus in oral fluid. *Open Forum Infect Dis*. 2016;3(1): ofw031. doi:10.1093/ofid/ofw031
6. El-Nagel M, Heuck C, Appel W, Vandepitte J, Engbaek K, Gibbs W. Basics of quality assurance for intermediate and peripheral laboratories. WHO Regional Office for the Eastern Mediterranean; 1992. Available from: <https://apps.who.int/iris/bitstream/handle/10665/120053/dsa90.pdf>. Accessed February 26, 2022.
7. Hunt L, Gupta-Wright A, Simms V, et al. Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study. *Lancet Infect Dis*. 2015;15(11):1292–1299. doi:10.1016/S1473-3099(15)00144-9
8. Scott JT, Sesay FR, Massaquoi TA, Idriss BR, Sahr F, Semple MG. Post-Ebola syndrome, Sierra Leone. *Emerg Infect Dis*. 2016;22(4):641–646. PMID: 26983037; PMCID: PMC4806950. doi:10.3201/eid2204.151302
9. Clark D, Jahrling P, Lawler J. Clinical management of filovirus-infected patients. *Viruses*. 2012;4(9):1668–1686. doi:10.3390/v4091668
10. Qureshi A. Clinical manifestations and laboratory diagnosis of Ebola virus infection. *Ebola Virus Dis*. 2016;117–138. doi:10.1016/B978-0-12-804230-4.00009-1
11. Clark DV, Kibuuka H, Millard M, et al. Long-term sequelae after Ebola virus disease in Bundibugyo, Uganda: a retrospective cohort study. *Lancet Infect Dis*. 2015;15(8):905–912. doi:10.1016/S1473-3099(15)70152-0

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Genotyping Approach to Predict Co^a and Co^b Antigens in Thai Blood Donor Populations

Oytip Nathalang, Kamonchanok Asisathaporn, Kamphon Intharanut, Wanlapa Chaibangyang, Nipapan Leetrakool, Supattra Mitundee & Sasitorn Bejrachandra

To cite this article: Oytip Nathalang, Kamonchanok Asisathaporn, Kamphon Intharanut, Wanlapa Chaibangyang, Nipapan Leetrakool, Supattra Mitundee & Sasitorn Bejrachandra (2023) Genotyping Approach to Predict Co^a and Co^b Antigens in Thai Blood Donor Populations, Journal of Blood Medicine, , 133-140, DOI: [10.2147/JBM.S398720](https://doi.org/10.2147/JBM.S398720)

To link to this article: <https://doi.org/10.2147/JBM.S398720>



© 2023 Nathalang et al.



Published online: 17 Feb 2023.



Submit your article to this journal



Article views: 201



View related articles



View Crossmark data

Genotyping Approach to Predict Co^a and Co^b Antigens in Thai Blood Donor Populations

Oytip Nathalang¹, Kamonchanok Asisathaporn¹, Kamphon Intharanut¹, Wanlapa Chaibangyang¹, Nipapan Leetrakool², Supattra Mitundee³, Sasitorn Bejrachandra⁴

¹Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand; ²Blood Bank Section, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; ³Regional Blood Centre 12th Songkhla, Thai Red Cross Society, Songkhla, Thailand; ⁴National Blood Centre, Thai Red Cross Society, Bangkok, Thailand

Correspondence: Oytip Nathalang, Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, 99 Moo 18 Klongluang, Pathumthani, 12120, Thailand, Tel +662-9869213 Ext. 7231, Fax +662-5165379, Email oytipnt@hotmail.com

Purpose: Co^a and Co^b antigens of the Colton (CO) blood group system are implicated in acute and delayed hemolytic transfusion reactions (HTRs). Owing to the inadequate supply of specific antiserum, data on CO phenotypes remain limited. This study aimed to develop genotyping methods to predict Co^a and Co^b antigens and to estimate transfusion-induced alloimmunization risks in three Thai blood donor populations.

Materials and Methods: The study included 2451 blood samples from unrelated healthy Thai blood donors obtained from central, northern, and southern Thailand. DNA sequencing was used to determine the *CO*A* and *CO*B* alleles. In-house PCR with sequence-specific primers (PCR-SSP) and high-resolution melting curve (HRM) assays were performed and genotyping results were compared using DNA sequencing. *CO*A* and *CO*B* allele frequencies among Thais were determined using PCR-SSP and their frequencies were compared with other populations. The risks of Co^a and Co^b transfusion-induced alloimmunization among Thai donor populations were calculated.

Results: The validated genotyping results by PCR-SSP and HRM assays agreed with DNA sequencing. The *CO*A/CO*A* was the most common (100.0, 100.0, and 99.3%), followed by *CO*A/CO*B* (0.0, 0.0, and 0.7%) among central, northern and southern Thais. Homozygous *CO*B/CO*B* was not found. The *CO*A* and *CO*B* allele frequencies among central Thais significantly differed compared among southern Thais ($p < 0.01$) but not among northern Thais. Those allele frequencies among Thais were similar to those of Taiwanese, Chinese and Malay-Malaysian populations but not to South Asian, Southeast Asian, Korean, Japanese, Filipino, French Basque, and Maltese populations ($p < 0.01$). A higher risk of anti-Co^b production rather than anti-Co^a production was particularly noted in the southern Thai population.

Conclusion: This study constitutes the first to determine *CO*A* and *CO*B* genotypes using PCR-SSP and HRM assays among Thais and this finding would be beneficial in predicting alloimmunization risk and providing safe transfusions among Thais.

Keywords: the Colton blood group system, genotypes, PCR-SSP, HRM, Thais

Introduction

The Colton (CO) blood group system (ISBT No. 015) currently consists of four antigens; a high prevalence antigen, Co^a with its antithetical antigen, Co^b, and the other high prevalence antigens, Co3 and Co4.¹ The CO antigens are carried on red blood cell (RBC) water transporter, aquaporin-1 (AQP1), encoded by the *AQP1* gene located on chromosome 7p14.^{2,3} Regarding the frequency of Co^a and Co^b antigens in populations, Co(a+b-) is the most common phenotype, 91.4–100.0%, followed by Co(a+b+), 0.0–8.4%, and Co(a-b+), 0.0–0.2%. The Co(a-b-) is a rare phenotype.^{4–7} Anti-Co^a is implicated in both acute and delayed hemolytic transfusion reactions (HTRs), and mild to severe hemolytic diseases of the fetus and newborn (HDFN). In contrast, anti-Co^b, a rare antibody, has been involved in acute and delayed HTRs, while no case of HDFN has been reported.^{2–4}

Both anti-Co^a and anti-Co^b are commonly IgG and reactive by the indirect antiglobulin test (IAT), especially when using protease-treated RBCs.^{3,8} However, IgM anti-Co^a has been reported.^{3,4} To date, anti-Co^a and anti-Co^b are not commercialized and are only available in specialized reference laboratories. Moreover, some limitations to perform precise Co^a and Co^b antigen typing using serological techniques are due to the positive direct antiglobulin test (DAT) and positive autocontrol in frequent transfused patients.² On the other hand, patients with multiple alloantibodies or unidentified antibodies make it challenging to determine antibody specificities; as a result, additional panel cells with uncommon or low prevalence antigens such as Kp^a, Vel and Co^b are needed.⁹

Given that antiserum for CO phenotyping is unavailable, CO genotyping can help to select the most appropriate blood for patients with anti-Co^a or anti-Co^b. The Co^a/Co^b polymorphism arises from a single nucleotide variation (SNV) associated with the c.134C>T (rs28362692) missense variant encoding p.Ala45Val (alanine for Co^a and valine for Co^b) in exon 1 of the *AQP1* gene.^{2–4} Polymerase chain reaction with sequence-specific primers (PCR-SSP) was implemented to distinguish between *CO*A* and *CO*B* alleles to predict Co^a and Co^b antigens among unrelated French Basque and Maltese blood donors.^{10,11} Additionally, multiplex PCR has also been used to identify those alleles in Austrian blood donor populations.¹² Subsequently, commercially available high-throughput techniques for red cell genotyping have been performed only in reference laboratories due to the high cost of reagents and the required complex equipment.^{5,6,13}

In Thailand, low-throughput methods such as PCR-SSP and multiplex PCR were performed to predict extended blood group phenotypes in donor and patient populations, and the results were consistent with the serological testing.¹⁴ Recently, high-resolution melting curve (HRM) analysis has been implemented to reduce errors in interpretation and manual inspection for red cell genotyping using PCR-SSP.¹⁵ Therefore, this study aimed to develop genotyping methods to predict Co^a and Co^b antigens and to estimate transfusion-induced alloimmunization risks in three Thai blood donor populations.

Materials and Methods

Sample Size

This experimental cross-sectional study was approved by the Human Research Ethics Committee of Thammasat University (HREC-TUSc) Pathumthani, Thailand in accordance with the Declaration of Helsinki (COE No. 012/2565). Written informed consent was obtained from all individual participants. The study included 2451 samples of EDTA-anticoagulated blood from unrelated healthy Thai blood donors. In all, 1724 samples were from the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand. In addition, 300 samples were from the Blood Bank Section, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand and 427 samples were from the Regional Blood Centre, 12th Songkhla, Thai Red Cross Society, Songkhla, Thailand.

Genomic DNA, extracted from all subjects using a Genomic DNA extraction kit (REAL Genomics, RBCBioscience, Taipei, Taiwan), was then stored at –20°C until used for genotyping.

Sequence-Based Genotyping

The genomic DNA of 400 blood donors was sequenced to identify *CO*A* and *CO*B* alleles. Primers were designed using Primer3 and BLAST. A fragment of 150 bp containing SNV (c.134 C>T; p. Ala45Val) in exon 1 of the *AQP1* gene was obtained from PCR amplification of genomic DNA using SEQ-CO-150-F forward and SEQ-CO-150-R reverse primers (Table 1). For each PCR reaction, 2 µL of genomic DNA (50 ng/µL) was a template for PCR amplification as a starting amount in a total volume of 50 µL using 0.6 µM of both forward and reverse primers for each reaction. PCR was performed using 25 µL of 2X PCR reaction mixture (Phusion High-Fidelity PCR Master Mix, New England BioLabs, MA, USA) and 17 µL of sterile distilled water in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

PCR was performed under the following conditions. Initial denaturation was conducted at 95°C for 30 sec. The PCR program began with 10 cycles of 10 sec at 95°C and 60 sec at 69°C, then 20 cycles of 30 sec at 95°C, 60 sec at 62°C, and 30 sec at 72°C. The last step was a final extension for 5 min at 72°C. The PCR products were subjected to electrophoresis and were visualized under a blue-light transilluminator. Then the PCR products were purified using a gel extraction kit

Table 1 Sequences of the Primers for *CO**A and *CO**B Genotyping

Specificity	Primer	Primer Sequences (5'→3')	Product Size (bp)	Uses
CO	SEQ-CO-150-F	GCCACGACCCTCTTTGTCTT	150	PCR amplification, Sequencing, HRM
	SEQ-CO-150-R	GTGGCCACACTCTGCG		
CO *A	CO-A-F	GGGGAACAACCAGACGGC	225	PCR-SSP
	CO-225-R	CCTGAGAGGATGGCGGTG		
CO *B	CO-B-F	GGGGAACAACCAGACGGT	225	PCR-SSP
	CO-225-R	CCTGAGAGGATGGCGGTG		
HGH	HGH-F	TGCCTTCCCAACCATTCCCTTA	434	PCR-SSP
	HGH-R	CCACTCACGGATTCTGTTGTGTTTC		

Abbreviations: Bp, base pair; CO, Colton; HGH, human growth hormone; HRM, high-resolution melting curve; PCR, polymerase chain reaction; PCR-SSP, PCR-sequence-specific primer.

(GeneJET Gel Extraction Kit, Thermo Scientific, MA, USA), and eluted fragments were sequenced (U2Bio Sequencing Service, Bangkok, Thailand) using the abovementioned PCR primers.

PCR-SSP

To identify *CO**A and *CO**B alleles the in-house PCR-SSP technique was used. Primers and amplification conditions were designed (Table 1). Briefly, 1 µL of genomic DNA (50 ng/µL) was a template for PCR amplification as a starting amount in a total volume of 10 µL using 0.5 µM of CO-A-F and CO-225-R primers to detect *CO**A alleles, and 0.5 µM of CO-B-F and CO-225-R primers to detect *CO**B alleles. Co-amplification of the *human growth hormone (HGH)* gene using 0.6 µM of HGH-F and HGH-R primers was run as the internal control. The PCR was performed with 5 µL of 2 x PCR reaction mixture (OnePCR Plus, GeneDirex, Taiwan) in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA), using the identical abovementioned PCR conditions, and the sample was kept at 4°C. PCR products were subjected to electrophoresis at 100 volts on 1.5% agarose gel using 1X TBE buffer and were visualized under a blue-light transilluminator. The PCR product size of both *CO**A and *CO**B alleles was 225 bp, whereas that of the internal control, the *HGH* gene, was 434 bp. Known DNA controls of *CO**A/*CO**A, and *CO**A/*CO**B genotypes were used as positive and negative controls in parallel with unknown samples. Altogether, 2451 DNA samples (including 400 samples tested by DNA sequencing), were used to detect *CO**A and *CO**B alleles using PCR-SSP. Additionally, the DNA samples from 100 randomly genotyped blood donors were retested by PCR-SSP with identical conditions as the first round testing and sequenced to validate our in-house PCR-SSP technique for CO genotyping.

HRM Assay

The HRM assay was developed for CO genotyping in 400 DNA samples confirmed by DNA sequencing. Each pair of forward and reverse primers SEQ-CO-150-F and SEQ-CO-150-R was designed to amplify the CO genes (Table 1), and 150 bp PCR products were expected. A DNA typing kit (Type-it HRM PCR Kit, QIAGEN GmbH, Valencia, CA, USA) was used to perform the PCR-HRM assay.

In a PCR reaction mix, 10 µL of 2X HRM PCR Master Mix reagent, 1 µL of genomic DNA (10 to 50 ng/µL concentration), and 0.35 µM of both forward and reverse primers were combined with water to make a final volume of 20 µL. PCR reaction tubes were placed on a real-time PCR cycler (Rotor-Gene Q 5plex HRM, QIAGEN GmbH, Valencia, CA, USA). The cycling parameters for the PCR program consisted of initial denature at 95°C for 5 min, followed by 40 cycles at 95°C for 30 sec, 62°C for 40 sec and 72°C for 30 sec. At the end of the PCR step, the temperature was gradually increased by 0.1°C every 2 sec from 65°C to 95°C. Total fluorescence and the rate of change in fluorescence were monitored, and data were acquired on the green and HRM channels. Rotor-Gene Q Series Software was used to analyze

the HRM data. Unknown melting profiles of DNA samples were compared with a set of determined controls on an allele-target basis. A confidence threshold of 90% was set for HRM genotyping calls.

To increase the validity and reliability of the evaluation, technicians were blinded to the PCR-SSP results. DNA sequencing was performed in case any discrepant genotyping results were obtained using the HRM and PCR-SSP assays.

Statistical Analysis

The gene and allele frequencies among the Thai blood donors were calculated using the gene-counting method. The chi-squared (χ^2) test was used to evaluate whether the observed genotype frequencies agreed with the expected ones under the Hardy-Weinberg equilibrium. The two-tailed Fisher's exact test of homogeneity was used to determine the differences of their allele frequencies among central, northern and southern Thais and to compare the differences with those reported in other populations.^{6,7,10,11,16,17} A *p*-value of less than 0.05 was considered statistically significant.

Results

Sequencing studies on 400 DNA Thai blood donor samples that exhibited the *CO***A* allele revealed the presence of c.134C, while the c.134T represented the *CO***B* allele. The two patterns of sequencing chromatograms are shown in Figure 1. The distribution of genotyping results among 400 DNA samples revealed that 398 were homozygous *CO***A*/*CO***A*, and 2 were heterozygous *CO***A*/*CO***B*. Homozygous *CO***B*/*CO***B* was not found in this group.

The results of the PCR-SSP were used to distinguish between the *CO***A* and *CO***B* alleles. The first and second mixes could identify the *CO***A* and *CO***B* alleles with an amplified product size of 225 bp, as shown in Figure 2. The validated genotyping results by PCR-SSP among DNA controls (*CO***A*/*CO***A* and *CO***A*/*CO***B*) were consistent with each other, and the results in both sets showed 100% concordance with 400 known DNA sequencing results. In addition, repeated

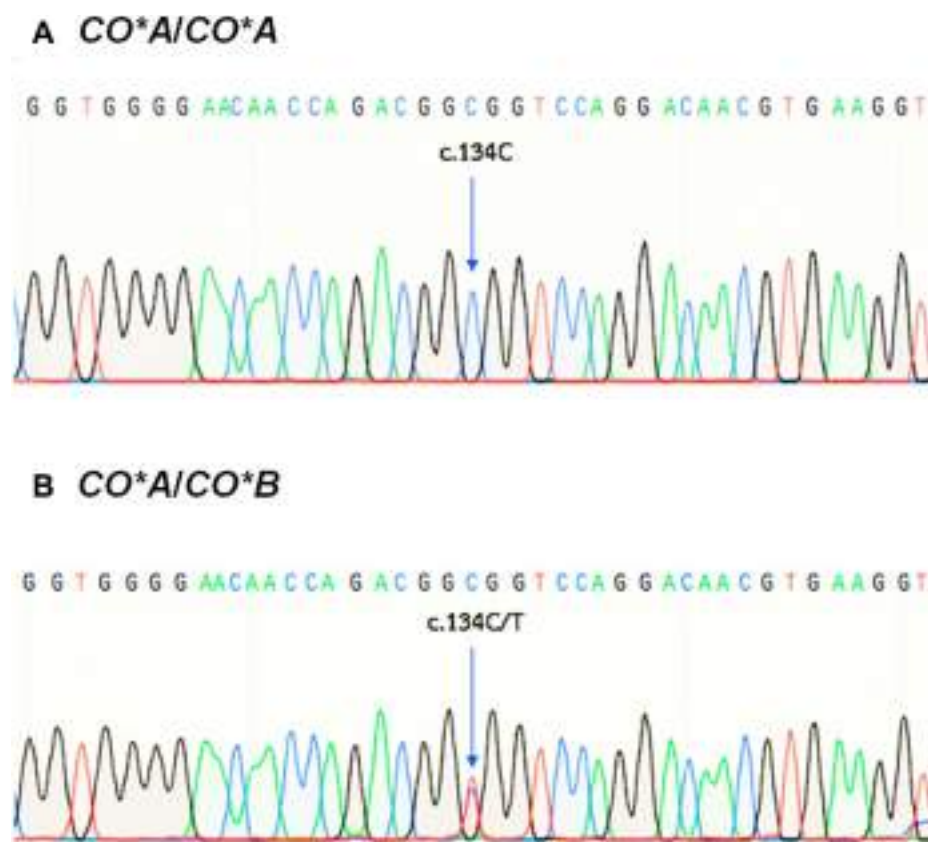


Figure 1 Electropherograms of the AQP1 Gene at the *CO***A* and *CO***B* Polymorphism Region, SNV c.134C>T (rs28362692). (A) Homozygous state of the c.134C identified in the *CO***A*/*CO***A* genotype and (B) Heterozygous state of the c.134C/T identified in the *CO***A*/*CO***B* genotype.

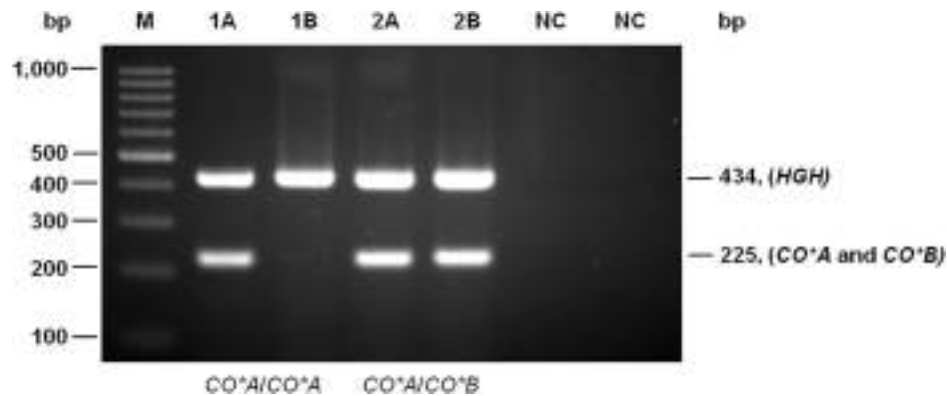


Figure 2 Representative Gel Shows Genotyping Results of the *CO*A* and *CO*B* Alleles Using the PCR-SSP Technique. The 434 bp amplification product of the HGH control primers was present in all lanes, showing that amplification had occurred optimally. The genotype was deduced from the presence or absence of 225 bp amplification product specific for *CO*A* and *CO*B* alleles. Lanes A and B referred to the specific *CO* allele test (*CO*A* and *CO*B*, respectively). From left to right: Lanes 1A-1B (SM101) = *CO*A/CO*A*; 2A-2B (SM106) = *CO*A/CO*B* and NC = negative control, respectively. M: 100 bp ladder marker (Fermentas, Carlsbad, CA, USA).

PCR-SSP was performed using 100 random DNA samples and confirmed by DNA sequencing. Those results of repeated testing were identical to the first round of testing and agreed with DNA sequencing.

Altogether, 1724, 300, and 427 samples obtained from central, northern and southern Thai blood donors, respectively, were genotyped for *CO*A* and *CO*B* separately, using the developed PCR-SSP (Table 2). *CO*A/CO*A* was found to be the most common genotype in the three groups of Thai blood donors (100.0, 100.0, and 99.3% in central, northern and southern Thais, respectively), while *CO*A/CO*B* was found only in southern blood donors (3/427, 0.7%); *CO*B/CO*B* was not found in the three groups. The allele frequencies of *CO*A* in central, northern and southern Thais were 1.000 (3448/3448), 1.000 (600/600), and 0.996 (851/854), respectively. In addition, the frequencies of *CO*B* were 0.000 (0/3448), 0.000 (0/600), and 0.004 (3/854), respectively. The genotypes of the 1724 central, 300 northern and 427 southern Thais were consistent with each other according to the Hardy-Weinberg equilibrium. The detected *CO*A* and *CO*B* allele frequencies among Thai blood donors significantly differed among central Thais compared with southern Thais ($p < 0.01$) but not among northern Thais (Table 2). Moreover, PCR-SSP was performed using 100 randomly selected samples consisting of 98 homozygous *CO*A/CO*A*, and two heterozygous *CO*A/CO*B*, confirmed by DNA sequencing. The results of the repeated testing were consistent with the DNA sequencing results.

DNA controls of *CO*A/CO*A* and *CO*A/CO*B* were amplified and analyzed using the HRM assay, while *CO*A* and *CO*B* alleles were genotyped using HRM curves. Representative normalized HRM curves were of two types: the red curve representing the homozygous *CO*A/CO*A* genotypes and the purple curve representing the heterozygous *CO*A/CO*B* genotypes. HRM curves showed a clear separation in those two genotypes at T_m ranging from 86.5 to 87.5°C for the *CO* genotyping (Figure 3). The developed HRM assay was implemented in 400 donor samples to predict phenotypes

Table 2 *CO*A* and *CO*B* Genotype and Allele Frequencies Among Thai Blood Donors

Frequency	Central Thais (N = 1724)	Northern Thais (N = 300)	Southern Thais (N = 427)	Total (N = 2451)
Allele				
<i>CO*A</i>	3448 (1.0000)	600 (1.0000)	851 (0.9965)	4899 (0.9994)
<i>CO*B</i>	0 (0.0000)	0 (0.0000)	3 (0.0035)	3 (0.0006)
Genotype				
<i>CO*A/CO*A</i>	1724 (100.0)	300 (100.0)	424 (99.3)	2448 (99.9)
<i>CO*A/CO*B</i>	0 (0.0)	0 (0.0)	3 (0.7)	3 (0.1)
<i>CO*B/CO*B</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: Allele frequencies in bold differed from those among central Thai blood donors ($p < 0.05$).

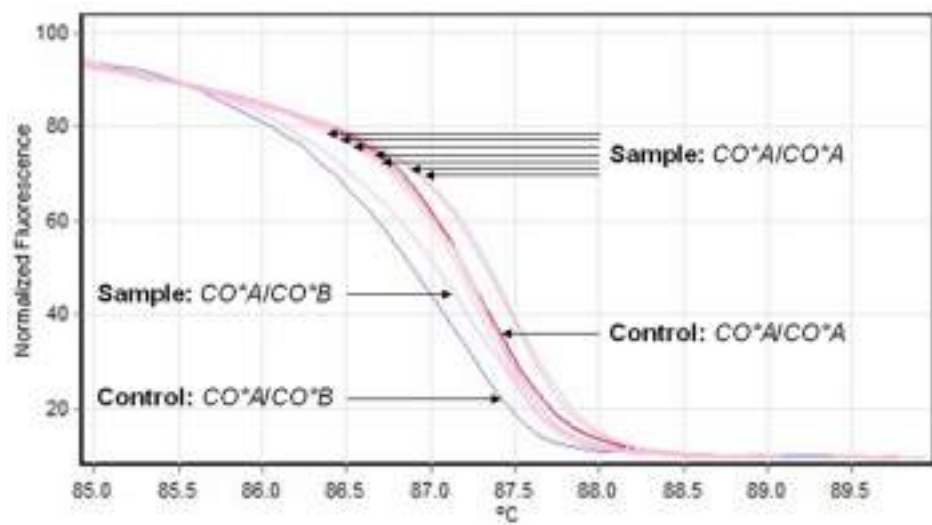


Figure 3 HRM analysis of CO genotypes.

of Colton blood group systems. The results were concordant with those 400 samples obtained for genotypes by DNA sequencing and PCR-SSP.

The *CO*A* and *CO*B* allele frequencies in Thai and other populations are shown in Table 3. The *CO*A* and *CO*B* frequencies among Thais were similar to previously reported in Thai,¹⁶ Taiwanese,¹⁷ Chinese⁶ and Malay-Malaysian⁷ populations ($p > 0.05$). Significant differences were observed when *CO*A* and *CO*B* allele frequencies among Thais

Table 3 Comparison of the *CO*A* and *CO*B* Allele Frequencies Among Different Populations

Population	Number of Alleles	Allele Frequency		p-value	Methods
		<i>CO*A</i>	<i>CO*B</i>		
Thai (current study)	4902	0.9994	0.0006	NA	PCR-SSP
Thai ¹⁶	790	0.9987	0.0013	0.45	MALDI-TOF MS
Thai ¹⁷	420	1.0000	0.0000	1.00	PCR-RFLP
Taiwanese ¹⁷	800	1.0000	0.0000	1.00	PCR-RFLP
South Asian ⁶	1844	0.9930	0.0070	< 0.0001	DNA array
Southeast Asian ⁶	1884	0.9965	0.0035	0.0066	DNA array
Chinese ⁶	3430	0.9985	0.0015	0.2867	DNA array
Korean ⁶	2066	0.9960	0.0040	0.0039	DNA array
Japanese ⁶	2044	0.9920	0.0080	< 0.0001	DNA array
Malay-Malaysian ⁷	194	0.9948	0.0052	0.1438	PCR-SSP
Filipino ⁶	2666	0.9950	0.0050	0.0002	DNA array
French Basque ¹⁰	222	0.9640	0.0360	< 0.0001	PCR-ASP
Maltese ¹¹	136	0.9412	0.0588	< 0.0001	PCR-ASPE

Note: Allele frequencies in bold differed from those among Thai blood donors ($p < 0.05$).

Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NA, Not applicable; PCR-ASP, polymerase chain reaction-allele-specific primer; PCR-ASPE, PCR-allele-specific primer extension; PCR-RFLP, PCR- restriction fragment length polymorphism; PCR-SSP, PCR-sequence-specific primer.

compared with South Asian,⁶ Southeast Asian,⁶ Korean,⁶ Japanese,⁶ Filipino,⁶ French Basque,¹⁰ and Maltese¹¹ populations ($p < 0.01$).

The risks of Co^a and Co^b alloimmunization in the three Thai populations were calculated, according to the observed phenotypes. Because the *CO*B/CO*B* genotype was not found in all Thai populations, only the alloimmunization risk of anti-Co^b production is supposed to be calculated. The *CO*A/CO*B* genotype was only observed in southern Thais; hence, the possibility of anti-Co^b production was 0.0069, estimated by *CO*A/CO*B* donor to *CO*A/CO*A* patient (0.9930×0.0070). Regarding regional groups, the risk of Co^b alloimmunization in southern Thailand was higher than in central and northern Thailand.

Discussion

For the Colton blood group system, anti-Co^a and anti-Co^b are required to determine the three CO phenotypes. To overcome the limitation of specific antisera, red cell genotyping is helpful in predicting phenotypes among blood donors and patients. Moreover, it remains impossible to identify various antibody specificities when employing reagent RBCs with only limited serologically antigen-typed information. A related study revealed the impact of using genotyped RBCs to identify antibody specificities. Altogether, 21 different antibodies from 10 different blood group systems including Knops, Yt, Vel, Dombrock, Lutheran, Rh, Diego, Colton, Scianna and Landsteiner-Wiener could be identified among 126 patients (1.5%) due to the genotyped information of the reagent RBCs.⁹

Red cell genotyping by commercially available Beadchip array has been used for assigning 34 antigens in 11 blood group systems in different populations.⁶ Nevertheless, some blood group antigens might only be significant in somewhat different populations and the reagents required are becoming costly. In this study, we developed the genotyping methods to determine *CO*A* and *CO*B* alleles, and PCR-SSP and HRM genotyping results agreed with DNA sequencing results; hence, the genotyping results were accurate and reproducible.

In addition, *CO* allele frequencies of Thai populations were compared with those reported of other groups.^{6,7,10,11,16,17} The significant difference in *CO*A* and *CO*B* frequencies among Thais compared with French Basque and Maltese populations might induce a higher probability of finding anti-Co^a in Co(a-b+) patients than in Thai patients. However, the rare donors, Co(a-b+) phenotype, still exist among those populations.^{10,11} A similar prevalence of predicted Co(a+) phenotype among Thais and Chinese was found in this study, corresponding to the Do(a+) phenotype in the Dombrock blood group system.¹⁸ Genotyping methods such as PCR-SSP and HRM are applicable to predict the phenotypes of extended blood group systems.^{14,15,19}

In Thailand, the Co^a/Co^b phenotyping is not routinely performed, and reagent red cells for antibody identification are also unspecified Co^a/Co^b antigens. In this study, the predicted Co^a and Co^b antigens were obtained by genotyping results, the highest prevalence was Co(a+b-) phenotype in all three Thai populations and Co(a+b+) donors were only observed in southern Thais. The possibility of anti-Co^b production caused by blood transfusion is high, especially among southern Thais compared with central and northern Thais. However, a related study of frequencies and antibody specificities among alloimmunized southern Thai patients revealed that more than 5% were undetermined antibody specificities.²⁰ Anti-Co^b may therefore hide in those patients owing to unspecified Co^a/Co^b antigens in panel cells. The genotyping in reagent RBCs, in particular Co(b+), is crucial to identify antibody specificity to prevent adverse transfusion reactions and to screen appropriate blood donors among patients with either anti-Co^a or anti-Co^b. Although the Co(a-b+) phenotype was not observed in this study, a patient with this phenotype produces an anti-Co^a, autologous blood donation, and recruiting related donors will be necessary.

Conclusion

This study constitutes the first to determine *CO*A* and *CO*B* genotypes in Thai populations using in-house PCR-SSP and HRM assays. This finding would be beneficial in predicting the risk of alloimmunization, especially anti-Co^b production, and providing safe transfusions among Thais.

Acknowledgments

This project is funded by the National Research Council of Thailand (NRCT), Bangkok, Thailand (Contract No. N35A650827). No external funding or donations were provided for this research.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. International Society of Blood Transfusion. Red cell immunogenetics and blood group terminology; 2022. Available from: <https://www.isbtweb.org/resource/tableofbloodgroupantigenswithinsystems.html>. Accessed November 4, 2022.
2. Cohn CS, Delaney M, Johnson ST, Katz LM. *Technical Manual*. 20th ed. Bethesda, MD: AABB; 2020:375.
3. Daniels G. *Human Blood Groups*. 3rd ed. Malden, MA: Blackwell Science; 2013:384–390.
4. Halverson GR, Peyrard T. A review of the Colton blood group system. *Immunohematology*. 2010;26(1):22–26. doi:10.21307/immunohematology-2019-196
5. Hong YJ, Chung Y, Hwang SM, et al. Genotyping of 22 blood group antigen polymorphisms and establishing a national recipient registry in the Korean population. *Ann Hematol*. 2016;95(6):985–991. doi:10.1007/s00277-016-2645-7
6. Delaney M, Harris S, Haile A, Johnsen J, Teramura G, Nelson K. Red blood cell antigen genotype analysis for 9087 Asian, Asian American, and Native American blood donors. *Transfusion*. 2015;55(10):2369–2375. doi:10.1111/trf.13163
7. Hajar CGN, Zefarina Z, Md Riffin NS, et al. Extended blood group profiles for Malays, Chinese, and Indians in Peninsular Malaysia. *Egypt J Med Hum Genet*. 2020;21:51. doi:10.1186/s43042-020-00096-y
8. Issitt PD, Anstee DJ. *Applied Blood Group Serology*. 4th ed. Durham, NC: Montgomery Scientific Publications; 1998:695–702.
9. Scharberg E, Rink G, Portegys J, et al. The impact of using genotyped reagent red blood cells in antibody identification. *Transfus Med Hemother*. 2018;45(4):218–224. doi:10.1159/000491884
10. Touinssi M, Chiaroni J, Degioanni A, et al. DNA-based typing of Kell, Kidd, MNS, Dombrock, Colton, and Yt blood group systems in the French Basques. *Am J Hum Biol*. 2008;20(3):308–311. doi:10.1002/ajhb.20720
11. Sutton G, Debono J, Borg J. Determining the frequency of Colton blood group antigens Co^a and Co^b in the Maltese population. *Malta J Health Sci*. 2019;6(2):6–15.
12. Jungbauer C, Hobel CM, Schwartz DW, Mayr WR. High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors. *Vox Sang*. 2012;102(3):234–242. doi:10.1111/j.1423-0410.2011.01542.x
13. Ji Y, Wen J, Veldhuisen B, et al. Validation of the multiplex ligation-dependent probe amplification assay and its application on the distribution study of the major alleles of 17 blood group systems in Chinese donors from Guangzhou. *Transfusion*. 2017;57(2):423–432. doi:10.1111/trf.13940
14. Nathalang O. Red cell genotyping in Thailand: trends for blood transfusion in Asian populations. *VOXS*. 2020;15:310–314. doi:10.1111/voxs.12547
15. Nathalang O, Intharanut K, Chidtrakoon S. High-resolution melting curve analysis to predict extended blood group phenotypes among Thai donors and patients. *Transfus Med Hemother*. 2021;49(3):163–171. doi:10.1159/000520713
16. Jongruamklang P, Gassner C, Meyer S, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of 36 blood group alleles among 396 Thai samples reveals region-specific variants. *Transfusion*. 2018;58(7):1752–1762. doi:10.1111/trf.14624
17. Peng CT, Tsai CH, Lee HH, Lin CL, Wang NM, Chang JG. Molecular analysis of Duffy, Yt and Colton blood groups in Taiwanese, Filipinos and Thais. *Kaohsiung J Med Sci*. 2000;16(2):63–67.
18. Wangmo T, Intharanut K, Leetrakool N, Mitundee S, Bejrachandra S, Nathalang O. Development of DO*A and DO*B allele detections to predict transfusion-induced alloimmunization risks in Thai populations. *Clin Lab*. 2021;67(10). doi:10.7754/Clin.Lab.2021.201220
19. Tanaka M, Takahashi J, Hirayama F, Tani Y. High-resolution melting analysis for genotyping Duffy, Kidd and Diego blood group antigens. *Leg Med*. 2011;13(1):1–6. doi:10.1016/j.legalmed.2010.08.004
20. Promwong C, Siammai S, Hassarin S, et al. Frequencies and specificities of red cell alloantibodies in the Southern Thai population. *Asian J Transfus Sci*. 2013;7(1):16–20. doi:10.4103/0973-6247.106718

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Optimizing Haemophilia Care in Resource-Limited Countries: Current Challenges and Future Prospects

Annick Ndoumba-Mintya, Yacouba L Diallo, Tagny C Tayou & Dora N Mbanya

To cite this article: Annick Ndoumba-Mintya, Yacouba L Diallo, Tagny C Tayou & Dora N Mbanya (2023) Optimizing Haemophilia Care in Resource-Limited Countries: Current Challenges and Future Prospects, Journal of Blood Medicine, , 141-146, DOI: [10.2147/JBM.S291536](https://doi.org/10.2147/JBM.S291536)

To link to this article: <https://doi.org/10.2147/JBM.S291536>



© 2023 Ndoumba-Mintya et al.



Published online: 20 Feb 2023.



Submit your article to this journal



Article views: 426



View related articles



View Crossmark data



Citing articles: 14 View citing articles

Optimizing Haemophilia Care in Resource-Limited Countries: Current Challenges and Future Prospects

Annick Ndoumba-Mintya¹, Yacouba L Diallo², Tagny C Tayou¹, Dora N Mbanya¹ 

¹Hematology & Transfusion Service, Yaoundé University Teaching Hospital, Yaoundé, Cameroon; ²Service de Médecine - CHU Hôpital du Mali, Bamako, Mali

Correspondence: Dora N Mbanya, Centre Hospitalier et Universitaire, B.P. 8046, Yaoundé, Cameroon, Tel +237 677607037, Email dmbanya1@yahoo.co.uk

Abstract: About 75% of persons with hemophilia live in the developing world and do not have access to routine care due to many barriers. There are a lot of challenges associated with hemophilia care in resource-limited settings, ranging from financial to organisational and government commitments. This review discusses some of these challenges and future prospects, while highlighting the important role of the World Federation of Hemophilia in hemophilia patient care. A participative approach involving all stakeholders is key to optimizing care in resource-limited settings.

Keywords: hemophilia care, people living with hemophilia, challenges, prospects

Introduction

Hemophilia is a rare bleeding disorder characterized by deficiency in clotting factors eight (VIII) for Hemophilia A and nine (IX) for Hemophilia B. Hemophilia A is predominant, and constitutes about 80–85% cases.¹ According to the World Federation of Hemophilia (WFH), there are an estimated 815,100 cases of hemophilia worldwide, of which only 347,026 are diagnosed, and with 276,900 cases being severe hemophilia.² Thus, it is generally underdiagnosed, especially in developing countries, where resources available for diagnosis and care are very restricted.

In developed countries, there is availability and accessibility of clotting factor concentrates (CFC), so much so that access to prophylaxis has been provided for over the last three decades. However, in developing countries, until recently, the only form of advanced care in persons living with hemophilia (PLWH) was the on-demand use of these CFCs when available. With other competing priorities such as malaria, malnutrition, and other infectious diseases, the tendency is not to focus much on chronic non-communicable diseases, especially in Sub-Saharan Africa. Thus, hemophilia care in these settings tends to be suboptimal. According to the World Federation of Hemophilia, 75% of persons with hemophilia live in developing countries,³ but have little access to routine care due to many challenges and barriers.

Evidence suggests that the incidence of hemophilia is constant in different populations; nevertheless, only about 2% of those requiring it actually use clotting factor concentrates for treatment in Africa.⁴ There is a huge disparity in capacities for prompt and accurate diagnosis and for the provision of the necessary care for survival in these settings. In Africa, only 11.4% of expected hemophilia cases are identified, with less than 0.1 IU/capita utilisation of clotting factor concentrates. This review touches on the general issues and challenges related to hemophilia care, and discusses perspectives for optimising care.

Care for People Living with Hemophilia (PLWH) Diagnosis

Care for PLWH begins with appropriate diagnosis. There is a huge difference in hemophilia diagnosis and care between the developed world and resource-limited settings, whereas in Europe and the Americas, 81% and 54% cases, respectively, are diagnosed, only 8% are diagnosed in Africa, and 15% in South-East Asia.⁵ And even within Africa, there is a lot of disparity and care of PLWH. In countries including South Africa, Algeria and Egypt, more patients are diagnosed in comparison to

countries of sub-Saharan Africa.^{5,6} Early diagnosis is the norm in developed countries.⁷ Clinically, a bleeding disorder is suspected in the face of recurrent hemorrhage, but it must be confirmed through laboratory screening tests.

The collaboration and support from the WFH has resulted in marked increase in the diagnosis of cases of PLWH, within their various communities. For example, in Cameroon, with an estimated prevalence of 2500 PLWH, only 225 (9%) have been diagnosed and followed up in the Hemophilia Treatment Center (HTC), a marked improvement on the 9 cases in 1972; the 65 cases in 2003; 166 cases in 2019 and 180 in 2021. Of the 225 cases, 185 (82.2%) are hemophilia A, 30 are Haemophilia B (13.3%) and the 10 others are FVII deficiencies (2 cases), and Von Willebrand Factor deficiencies (8 cases). There are 125 cases of severe hemophilia (55.5%) recorded.

In Mali, there are currently 220 diagnosed and registered patients in the Hemophilia Treatment Center (HTC), of which 150 are hemophilia A (68.2%); 17 are hemophilia B (7.7%), and 29 are simply suspected cases. The remaining 24 cases are constituted of Willebrand disease, and other rare bleeding disorders (10.9%). On the whole, there is significant evolution, given that only in recent years all samples from Mali were exported to foreign Centers for diagnosis. Today, both screening for hemophilia and inhibitors is carried out there. Nevertheless, the diagnosis of von Willebrand's Disease remains challenging for most countries in resource-limited settings.

Management

Once diagnosed, clinical care and follow-up is indispensable. The WFH recommends that care in PLWH be multi-disciplinary requiring hematologists, paediatricians, dentists, physiotherapists, rheumatologists, radiologists, nurse coordinators, psychologists, and more.^{8,9} Needless to emphasize that there must be harmony between these team members, the HTC, the patients and their families, as well as a functioning laboratory for clotting factor assays and screening for inhibitors. In addition, proper records/registry should be kept on patient management for proper follow-up. Continuous patient and family education also contributes significantly to proper care for PLWH.⁹

Although blood and labile blood products are still a scarce commodity, transfusion therapy has been the mainstay of treatment since the 1940s, with mainly whole blood used. Plasma was used by the 1950s and cryoprecipitate by the mid 1960s. In the late 1960s, scientists and manufacturers developed methods for separating factor VIII and factor IX from pooled human plasma. Freeze-dried (lyophilized) factor VIII or factor IX concentrates were prepared in bottles and widely used in the developed world. However, in the 1980s, human blood, plasma, and plasma-derived products were discovered to be transmitting deadly blood-borne viruses (HIV, HBV, HCV) so much so that many PLWH were infected by these agents, and most died of AIDS or end-stage liver disease.

In 1984, the successful cloning of the factor VIII gene was a major breakthrough that led to the production of recombinant human factor VIII (r FVIII). Clinical trials were ongoing by 1987 and by 1992 two pharmaceutical companies had licensed rFVIII products for use in hemophilia A. Five years later, in 1997, rFIX (BeneFIX) became available for people living with hemophilia B.

In recent years treatment options have improved to include the treatment of inhibitors to FVIII or FIX as well as prophylaxis. Nevertheless, transfusion of blood and blood components remain the mainstay of managing bleeding patients in most resource-limited settings. For example, in Mali, before 2012, the treatment of bleeding events was only with fresh frozen plasma transfusions (Yacouba Diallo, personal communication). Yet blood safety challenges remain multifactorial in these settings.¹⁰ Hence, complications related to blood transfusion remain real in these settings. The transmission of infections by transfusion (TTIs) is a pending threat because of the high prevalence of HIV, hepatitis B and C viruses, syphilis and malaria in these populations, and the lack of resources for their appropriate screening. More recent technologies for reducing the transmission of these pathogens through blood transfusion, such as pathogen reduction/inactivation techniques are costly and remain inaccessible to most of these countries.

Thus, the evolution in care for PLWH in the developing world has gone from the use of large quantities of whole blood, which in itself is a scarce commodity, to the use of fresh frozen plasma, not readily available, to that of cryoprecipitate, also not usually available. The latest WHO report on blood availability, safety and quality in the African Region,¹¹ indicates that only a few countries of the AFRO Region are actively involved with component preparations. Only 7 countries reported using apheresis, while red cell concentrates use was reported by 73.9%; platelet concentrates by 9.5%, and cryoprecipitate by 0.6%.¹¹

The Twinning programs organized by the WFH between experienced Hemophilia Treatment Centers and emerging ones have been very successful. This program allows for exchange of experiences between the two centers and capacity building in favor of the emerging centers and has also contributed significantly in promoting care in resource-limited settings. Currently, with the collaboration of World Federation of Hemophilia's Humanitarian Aid Program, there is increased access to factor VIII and IX concentrates, and marked improvement in care for PLWH. Access to other novel therapies has revolutionized hemophilia care.¹² For example, the use of Emicizumab, a humanized bispecific monoclonal antibody that bridges activated FIXa and FX to restore the function of missing FVIIIa, has become accessible to selected patients in some low-income countries, through the WFH Humanitarian Aid Program. Several beneficiaries have had remarkable improvement in their quality of life with reduced annualized bleeding rates and decreased morbidity and mortality rates.

A review by Ghosh and Ghosh¹³ suggests that for care to be optimal the following should be taken into consideration: the number of persons living with hemophilia; minimum amount of concentrate required to keep a PLWH relatively free of bleeds; strategies for sustainable accessibility of clotting factors for PLWH, and ways to minimize and optimize their usage.

The organization of hemophilia care delivery in many African countries is at its early stages. Thus, there is still high morbidity and mortality due to hemophilia, with rampant occurrence of chronic complications and sequelae.

Challenges

Hemophilia care in resource-limited settings is plagued with various challenges that have also been reported by several authors. These include the lack of proper health care infrastructure (diagnostic laboratories and appropriate management centers), and lack of human resources trained for hemophilia care among others.⁶ And the challenges are similar in other countries like Nigeria.¹⁴ In most low-income settings, even establishing one HTC is a great achievement. In Cameroon for example, there is one in the capital city, and 2 satellite centers in two other cities. In 2017, their analysis of the Quality of Life (QoL) in PLWH in the HTC in Yaoundé, and despite not meeting the ideal international standards, Nzometia et al reported significant improvement in the QoL of those using the facility.¹⁵ Nevertheless, a few HTCs including that of Senegal in Dakar have both improved diagnosis, care and capacity building of care personnel.¹⁶ This HTC has evolved to become a training center on hemophilia care, and hosting several workshops and seminars.

The use of national registries for data collection (type of bleeding disorder; treatment and doses, complications etc.) and follow-up of PLWH, also contributes not only to enhance care, but serves as a good tool for advocacy. These exist in countries like Egypt.⁶ In other resource-limited settings, the tendency is to have a register only at the HTC. Today, the WFH has facilitated the creation of national registries in about fifty countries.

Other challenges include the fact that these settings have several competing health care priorities for the government including infectious disease, so that disorders like hemophilia get relegated to the background. Interestingly, the Senegalese government has prioritized hemophilia in its list of chronic diseases.⁶ Furthermore, there is lack of visibility of hemophilia patients in healthcare system including low awareness of the disorder within the medical profession, the population and the policy makers. The non-availability of clotting factor concentrates; their inadequate utilization when available, and challenges related to the development of inhibitors all hamper care for PLWH. Two studies in Cameroon reported the presence of inhibitors in PLWH on CFCs.^{17,18} The lack or complete absence of social security and medical insurances are all factors that further jeopardize hemophilia care in resource-limited settings.

Future Prospects

Developing a patient's society (National Members Organisation) and a bleeding disorder registry are very important steps towards ensuring adequate treatment for PLWH in resource-limited settings, as is the case in developed countries. The World Federation of Haemophilia has been instrumental in promoting these in several countries. These patient associations collaborate with the WFH as associate members. They participate actively in sensitizing and mobilising their respective communities towards haemophilia care. The Haemophilia Association of Cameroon was created in 2003 with a handful of members. Today, they count about 216 members. In Mali, their association was created in 2012 and counts about 218 members. As part of a four-year training program supported by the Novo Nordisk Hemophilia Foundation, the Malian association contributes significantly in awareness activities, the organization of training sessions involving patients, their families, medias workers, opinion leaders, health authorities and health care providers including both modern and traditional practitioners, through all regions of Mali (Prof. Yacouba Diallo,

personal communication). Furthermore, special training sessions for Laboratories technicians and biologists have been included in their activities.

Table 1 is a summary of strategies that may impact positively on haemophilia care in these settings. Government commitment should be sought, to enhance their allocation of budgets for hemophilia care. Strategies for promoting the use of safe plasma products through quality management systems and Good Manufacturing Procedures; it will avoid wastages and provide for needs. In Egypt, despite introducing hemophilia care since the late sixties, and despite heavy support from their government, they still face challenges with procurement of CFCs to be able to align with universal prophylaxis and home therapy (Mbanya et al, 2021). A good supply chain management is necessary for improved care.

Establishing a good supply chain management for accelerating the use of plasma-derived medicinal products (PDMPs) in resource-limited settings could increase access to CFCs. Indeed, it has been reported that 20 of 45 countries of the WHO-AFRO Region (43.5%) had included PDMPs on their national lists of Essential Medicines. These, if accessible, would be safer for use in these settings, than plasma and cryoprecipitate.

However, despite the great need for these products, there is a lot of plasma wastage in these settings. But it was equally obvious that several basic requirements are lacking for the local production of PDMPs, including insufficient plasma collections from healthy, voluntary non-remunerated, repeat, blood donors (most donors in these settings are family/replacement donors), and wastage of available plasma. The non-conformities with international guidelines and regulations for testing and issuing of plasma suitable for fractionation and the absence of well-regulated national blood services, are other issues. In a recent concept note by the World Health Organisation,¹⁹ the steps that can be taken to increase the production of quality and safe plasma from voluntary non-remunerated donors in low- and medium-income countries, useable in fractionation, were provided. This guideline is intended to provide a high-level overview and recommendations for actions that can be taken by policy-makers, national regulatory authorities, blood collection organizations, blood donors and their associations, clinicians and patients as a roadmap toward reducing wastage of plasma that could be fractionated to make the PDMPs and thus increase access to PDMPs in low- and middle-income countries.

The coordinated delivery of comprehensive care by multidisciplinary teams of healthcare professionals should be encouraged.

Primary prophylaxis with low/intermediate dose of the concentrates is a cost-effective way to manage PLWH and this also reduces inhibitor development. Strategies to encourage adherence to therapy will further enhance care in these settings. Promoting regular physiotherapy and dental care are aspects of care for PLWH to be fostered in resource-limited settings. Home therapy is another prospect to examine.

Epidemiological and community-based studies should be promoted, for improved diagnosis of PLWH, as well as the introduction of antenatal diagnostic centres.

Table 1 Strategies for Improving Haemophilia Care in RLS

Strategy	Impact on Care
Government commitment	Enhances budget allocation toward hemophilia care
Promote use of safe plasma products through quality management systems and Good Manufacturing Procedures	Will provide for needs and minimize wastages
Good supply chain management for accelerating the use of plasma-derived medicinal products (PDMPs)	Increased access to CFCs
Use of multidisciplinary teams of healthcare professionals	More coordinated delivery of comprehensive care
Primary prophylaxis with low/intermediate doses of concentrates	A cost-effective way of management, minimizing the development of inhibitors
Adherence to therapy	Better health
Epidemiological and community-based studies	Enhance diagnosis of more PLWH

Gene therapy is showing promising results, but there may be a number of barriers to access. The WFH has coordinated many initiatives, in partnership with private laboratories, to provide donations of Extended half-life CFC and Emicizumab to selected patients in resource-limited countries.²⁰ However, it is very important for other stakeholders (governments, physicians, patient organisations), work together to facilitate further access to these products.

Conclusions

The care of PLWH is plagued with many challenges and while there are also many prospects towards tackling them, concerted efforts are indispensable between all stakeholders (policy-makers, national regulatory authorities, blood collection organizations, patient associations and the international community), for these to be overcome. Unfortunately, haemophilia and other non-communicable diseases may not be considered a healthcare priority (a national health problem), in comparison to malaria, HIV/AIDS and other infectious diseases in these our settings. Awareness campaigns and advocacy becomes indispensable.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Stonebraker JS, Bolton-Maggs PH, Michael Soucie J, Walker I, Brooker M. A study of variations in the reported haemophilia B prevalence around the world. *Haemophilia*. 2012;18:91–94. doi:10.1111/j.1365-2516.2011.02588.x
2. World Federation of Hemophilia. Nigeria includes bleeding disorders in national policy and strategic plan. Available from: <https://wfh.org/nigeria-includes-bleeding-disorders-in-national-policy-and-strategic-plan/>. Accessed February 2, 2023.
3. World Federation of Hemophilia. Economic benefits of basic hemophilia management and care; 2004. Available from: <http://www.wfh.org>. Accessed February 2, 2023.
4. World Federation of Hemophilia. *Report on the Annual Global Survey 2019*. Montreal: WFH; 2020.
5. World Federation of Hemophilia. *Report on the Annual Global Survey 2020*. Montreal: World Federation of Hemophilia; 2021.
6. Mbanya DN, Diop S, Ndoumba Mintya AN, et al. Hemophilia care in Africa: status and challenges. *Transfus Clin Biol*. 2021;28:158–162. doi:10.1016/j.tracbi.2021.01.008
7. Haute autorité de Sante. *Protocole National de Diagnostic Et de Soins (PNDS) Hemophilie*. France: Haute autorité de Sante; 2019.
8. Federation Mondiale de l'Hemophilie. *Lignes directrices pour la prise en charge de l'hemophilie*. Montreal: Federation Mondiale de l'Hemophilie; 2012.
9. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia. *Haemophilia*. 2020;00:1–158.
10. Tagny CT, Mbanya D, Tapko JB, Lefrère JJ. Blood safety in Sub-Saharan Africa: a multi-factorial problem. *Transfusion*. 2008;48:1256–1261. doi:10.1111/j.1537-2995.2008.01697.x
11. World Health Organisation. *African Region Status Report on Blood Availability, Safety and Quality*. Brazzaville: AFRO; 2022.
12. Weyand AC, Pipe SW. New therapies for hemophilia. *Blood*. 2019;133:389–398. doi:10.1182/blood-2018-08-872291
13. Ghosh K, Ghosh K. Overcoming the challenges of treating hemophilia in resource-limited nations: a focus on medication access and adherence. *Expert Rev Hematol*. 2021;14:721–730. doi:10.1080/17474086.2021.1957826
14. Okoye HC, Korubo KI, Nwogoh B, et al. Challenges in the management of bleeding disorders in Nigeria. *Niger J Clin Pract*. 2018;21:468–472. doi:10.4103/njcp.njcp_319_17
15. Nzometia CY, Tagny CT, Ndoumba AM, Balogog PN, Mbanya DN. Assessing the quality of care for haemophilia at the Yaoundé reference treatment Centre of Cameroon. *Blood Coagul Fibrinolysis*. 2017;28:176–180. doi:10.1097/MBF.0000000000000549
16. Diop S, Haffar A, Mahlangu J, Chami I, Kitchen S, Pierce G. Improving access to hemophilia care in sub-Saharan Africa by capacity building. *Blood Adv*. 2019;3:1–4. doi:10.1182/bloodadvances.2019GS121537
17. Balogog PN, Tagny CT, Ndoumba A, Mbanya D. FVIII and FIX inhibitors in people living with hemophilia in Cameroon, Africa: a preliminary study. *Int Jnl Lab Hem*. 2014;36:566–570. doi:10.1111/ijlh.12190
18. Chendjou A, Ndoumba A, Kalla C, Tayou C, Mbanya D. FVIII inhibitors during primary prophylaxis for hemophilia A: a pilot study in Cameroon. *J Blood Transfusions Dis*. 2020;3:126–129.
19. World Health Organisation. *Guidance on Increasing Supplies of Plasma-Derived Medicinal Products in Low- and Middle-Income Countries Through Fractionation of Domestic Plasma*. Geneva: World Health Organisation; 2021.
20. Pierce GF, Adediran M, Diop S, et al. Achieving access to haemophilia care in low-income and lower-middle-income countries: expanded humanitarian aid program of the world federation of hemophilia after 5 years. *Lancet Haematol*. 2022;9(9):e689–e697. PMID: 36055333. doi:10.1016/S2352-3026(22)00209-5

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Outcome of Primary Mediastinal Large B Cell Lymphoma Treated with RCHOP

Khalid Halahleh, Abeer Yaseen, Isra Muradi, Akram Al-Ibraheem, Iyad Sultan & Mohammad Ma'koseh

To cite this article: Khalid Halahleh, Abeer Yaseen, Isra Muradi, Akram Al-Ibraheem, Iyad Sultan & Mohammad Ma'koseh (2023) Outcome of Primary Mediastinal Large B Cell Lymphoma Treated with RCHOP, Journal of Blood Medicine, , 147-157, DOI: [10.2147/JBM.S393180](https://doi.org/10.2147/JBM.S393180)

To link to this article: <https://doi.org/10.2147/JBM.S393180>



© 2023 Halahleh et al.



Published online: 20 Feb 2023.



Submit your article to this journal



Article views: 300



View related articles



View Crossmark data

Outcome of Primary Mediastinal Large B Cell Lymphoma Treated with RCHOP

Khalid Halahleh¹, Abeer Yaseen¹, Isra Muradi², Akram Al-Ibraheem³, Iyad Sultan⁴,
Mohammad Ma'koseh¹

¹Department of Internal Medicine, King Hussein Cancer Center, Amman, Jordan; ²Department of Medicine, University of Tripoli, Tripoli, Libya;

³Department of Nuclear Medicine, King Hussein Cancer Center, Amman, Jordan; ⁴Department of Pediatric Oncology, King Hussein Cancer Center, Amman, Jordan

Correspondence: Mohammad Ma'koseh, Department of Internal Medicine, King Hussein Cancer Center, Queen Rania Al Abdullah Street, P.O. Box 1269, Amman, 11941, Jordan, Tel +962-6 5300460, Ext 1146, Email mm.09744@khcc.jo

Purpose: Primary mediastinal large B-cell Lymphoma (PMLBCL) is a rare aggressive lymphoma with unique clinical, pathological, and molecular features. The optimal frontline therapy is subject of ongoing debate. Our study aims to evaluate the outcomes of PMLBCL treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP) at King Hussein Cancer Center.

Patients and Methods: Adult patients >18 years of age with PMLBCL treated with RCHOP from January 2011 to July 2020 were identified. All demographics, disease and treatment related variables were retrospectively collected. Correlations of clinical and laboratory variables with progression-free survival (PFS) and overall survival (OS) were determined by univariate and multivariate analyses using backward stepwise Cox regression models. The PFS and OS were plotted using Kaplan–Meier curves.

Results: 49 patients were included with a median age of 29 years. 14 (28.6%) had stage III or IV, 31 (63.3%) had mediastinal bulky disease. International prognostic index (IPI) was 0–1 in 35 (71.4%). Radiotherapy was given to 32 (65.3%) patients. End of treatment (EOT) response was complete (CR) in 32 (65.3%), partial response (PR) in 8 (16.3%) and progressive disease (PD) in 9 (18.4%). Patients who achieved CR at EOT, compared favorably with those who did not in regard to 4-year OS (92.5% vs 26.9%, $p < 0.001$). Overall objective response to salvage chemotherapies was 26.7%. At a median follow-up of 46 months, 4-year PFS and OS were 60% and 71% respectively. In multivariate analysis, IPI > one correlated with the EOT response ($p = 0.009$), PFS ($p = 0.004$) and OS ($p = 0.019$).

Conclusion: In PMLBCL, RCHOP chemotherapy backbone in the frontline therapy is suboptimal but can be used in patients with low IPI. Adapting more intensive chemoimmunotherapy regimens may be considered for patients with high IPI. Salvage chemotherapy has limited activity in patients with relapsed or refractory disease.

Keywords: international prognostic index, end of treatment response, radiotherapy, salvage chemotherapy

Introduction

Lymphomas are relatively common tumors in Jordan with 485 reported cases in 2017 and ranked as the third most common tumor.¹ Primary mediastinal large B-cell lymphoma (PMLBCL) is a rather infrequent aggressive lymphoma with unique clinical, pathological, and molecular features, accounts for 2–4% of all non-Hodgkin lymphomas (NHL).^{2–8} The 2022 World Health Organization (WHO) classification of lymphoid neoplasms identified PMLBCL as a unique clinical and biological entity.⁹ In contrast to diffuse large B cell lymphoma (DLBCL), PMLBCL typically presents at a median age of approximately 35 years and the majority of patients present with an early-stage but bulky mediastinal disease.^{2,5,10}

The optimal therapeutic approach has not been defined due to the rarity of disease and absence of randomized controlled trials. In the pre-rituximab era, retrospective studies suggested that outcomes with more intensified approaches like, V/MACOP-B (etoposide or methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) are superior to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone).^{11,12} However, the addition of rituximab

to CHOP might mitigate this effect.¹³ Nonetheless, 20–25% of patients treated with rituximab in combination with anthracycline based chemotherapy had relapse or refractory disease.^{13–15}

Recently, dose adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and rituximab (DA-EPOCH-R) regimen and a sequential administration of four doses of RCHOP followed by three cycles of rituximab, Ifosfamide, carboplatin, and etoposide (R-ICE) resulted in favorable outcomes and obviated the need for consolidative radiotherapy (RT), but with higher treatment-related toxicities.^{16–19}

Our study aims to assess the outcomes of primary mediastinal large B cell lymphoma treated with RCHOP in our center.

Materials and Methods

We searched Cancer Registry of King Hussein Cancer Center (KHCC) for adults (age >18 years) with non-Hodgkin lymphoma, from January of 2011 to July 2020 (n=1156). Patients with a pathological diagnosis of PMLBCL treated with R-CHOP-21 with or without RT were identified and included. Pathological diagnosis was confirmed by the expression of B cell antigens (CD19, CD20, CD23, and CD79a) and the lack of CD15. CD30 positivity supported the diagnosis but was not required.

All demographics, laboratory, and treatment-related variables, including age, gender, date of diagnosis, Eastern Cooperative Oncology Group (ECOG) performance status, serum lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), beta-2 microglobulin (B2M), number of extranodal sites, disease stage and disease bulk were collected. Bulky disease is defined as a mediastinal mass of ≥ 10 centimeters.

Staging and response to treatment were determined according to Lugano criteria.²⁰ Initial staging was done with a computed tomography (CT) scan in 30 (61%) patients and with a CT scan and a positron emission tomography (PET)-CT scan in the remaining patients. Response to treatment was determined with PET-CT scan in 44 (89.7%) patients and with a CT scan only in 5 (10.3%) patients. International prognostic index (IPI) was calculated as previously described.²¹

Consolidative radiotherapy (RT) was given to patients with a tumor diameter >7 cm with no evidence of disease progression after finishing chemotherapy. Descriptive statistics were used to characterize patients at baseline and presented as counts and percentages. Overall survival (OS) was calculated from the date of diagnosis to the date of the last encounter or death from any cause. Progression free survival (PFS) was calculated from the date of diagnosis to the date of relapse or progression. Survival outcomes were calculated using the Kaplan Meier method. The associations between different variables with PFS and OS were analyzed using Log-rank (Mantel-Cox) test in the univariate analysis and Cox proportional hazard models in the multivariate analysis. Patients' characteristics and the end of treatment (EOT) response were compared using the Fisher's exact test. ECOG performance status was excluded from the analysis because one patient had an ECOG performance status of >1. The P-values are two-sided and considered significant when < 0.05. The waiver of the written informed consent was granted due to the retrospective nature of the review, and the data were anonymized and maintained with confidentiality. The study was approved by the King Hussein Cancer Center Institutional Review Board (IRB) complaint with the principles of the declaration of Helsinki.

Results

49 patients were included in the analysis with a median age of 29 (range:18–56) years and an almost equal gender distribution. 14 (28.6%) had stage III or IV, and 11 (22.4%) had two or more extranodal sites. CD30 was reported to be positive in 41 (83.7%) patients. About two thirds of patients had mediastinal bulky disease. IPI was 0–1 in 35 (71.4%). All patients received at least six cycles of RCHOP; 39 (79.6%) patients were given six cycles and 10 (20.4%) patients were given 7–8 cycles. Consolidative RT at doses between 30.6Gy to 39.6Gy was given to 32 (65.3%) patients, among which four (12.5%) patients were given involved site radiotherapy and 28 (87.5%) patients received involved field radiotherapy. Patients' characteristics are detailed in Table 1.

After chemotherapy completion, 39 (79.6%) had an objective response among which 30 (61.2%) had complete response (CR) and nine (18.4%) had partial response.

Consolidative RT was given to 22 (73.3%) patients while in CR, all continued to have CR at the last encounter and to 7 out of 9 patients while in PR; 2 of the moved to CR after RT, 2 PR and 3 had disease progression (DP). 3 of the 10

Table I Patients Characteristics and Treatment

Characteristic	Number (%)
Gender	
Male	27 (55%)
Female	22 (45%)
Median age (range)	29 (18–56) years
ECOG performance status	
0–I	48 (98%)
2 or more	1 (2%)
B symptoms	
Yes	29 (59%)
No	20 (41%)
Bulky disease (7 or more centimeters)	45 (92%)
Bulky disease (10 or more centimeters)	31 (63.3%)
Stage	
I	6 (12.2%)
II	29 (59.2%)
III	3 (6.1%)
IV	11 (22.4%)
Number of extranodal sites	
0	37 (75.5%)
I	3 (6.1%)
2 or more	9 (18.4%)
High LDH	36 (37.5%)
B2 microglobulin (2.5 mg/dl or more)	11 (22.4%)
ESR (50 mL/hour or more)	15 (30.6%)
IPI	
0–I	35 (71.4%)
More than I	14 (18.6%)
Number of RCHOP cycles	
6	39 (79.6%)
7 or 8	10 (20.4%)
Radiotherapy consolidation	32 (65.3%)
First line salvage treatment	19 (38.8%)
DHAP	10 (52.6%)
GDP	4 (21%)
ICE	3 (15.8%)
High dose methotrexate	2 (10.5%)
Second line salvage treatment	15 (30.6%)
ICE	10 (66.6%)
GDP	2 (13.3%)
DHAP	1 (6.6%)
R-Gem-Ox	1 (6.6%)
Mini-BEAM	1 (6.6%)

(Continued)

Table 1 (Continued).

Characteristic	Number (%)
Third line salvage treatment	9 (18.4%)
Pembrolizumab	2 (22.2%)
Mini-BEAM	3 (33.3%)
ICE	1 (11.1%)
GDP	1 (11.1%)
DHAP	1 (11.1%)
Hyper-CVAD	1 (11.1%)

Abbreviations: ESR, Erythrocyte sedimentation rate; LDH, Lactate dehydrogenase; IPI, international prognostic index; DHAP, cisplatin, cytarabine and dexamethasone; GDP, cisplatin, gemcitabine and dexamethasone; ICE, Ifosfamide, carboplatin and etoposide; R-Gem-Ox, rituximab, gemcitabine and oxaliplatin; Mini-BEAM, carmustine, etoposide, cytarabine and melphalan; Hyper-CVAD, cyclophosphamide, vincristine, dexamethasone alternating with cytarabine and methotrexate.

patients who progressed were given salvage RT, all progressed. Based on the decision of the treating physician, four patients with a bulky disease had a CR and two patients with a PR did not receive consolidative radiotherapy.

End of treatment (EOT) response to either chemotherapy or combined modality treatment revealed CR in 32 (65.3%), no CR in 17 (34.7%) [PR in 8 (16.3%) and DP in 9 (18.4%)], [Table 2](#).

Table 2 Response to Frontline and Salvage Treatments

Response to frontline chemotherapy	
Complete response	30 (61.2%)
Partial response	9 (18.4%)
Disease progression	10 (20.4%)
End of treatment response	
Complete response	32 (65.3%)
Partial response	8 (16.3%)
Disease progression	9 (18.4%)
Response to first line salvage chemotherapy (n=19)	
Complete response	1 (5.2%)
Partial response	1 (5.2%)
Disease progression	17 (89.6%)
Response to second line salvage chemotherapy (n=15)	
Partial response	2 (13.3%)
Disease progression or death	13 (86.7%)
Response to third line salvage chemotherapy (n=9)	
Complete response	2(22.2%)
Disease progression or death	7 (77.8)
ASCT	5 (10.2%)
Relapse post ASCT	
Yes	1 (20%)
No	4 (80%)
Status on last encounter	
Alive	35 (71.4%)
Dead	14 (28.6%)

Abbreviation: ASCT: autologous stem cell transplant.

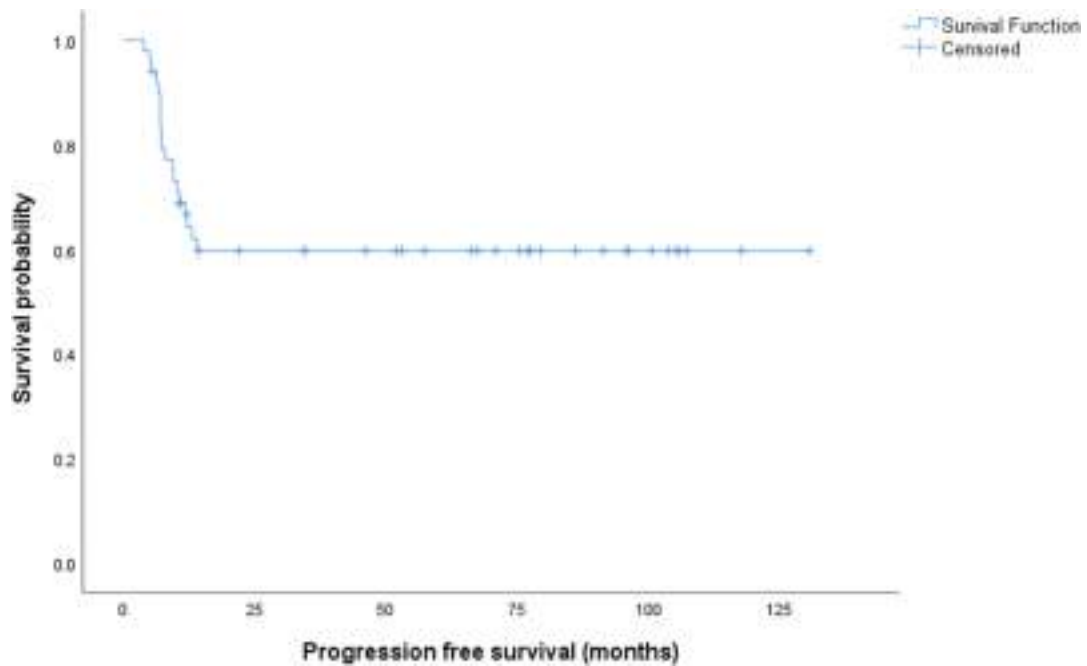


Figure 1 Progression free survival in all patients.

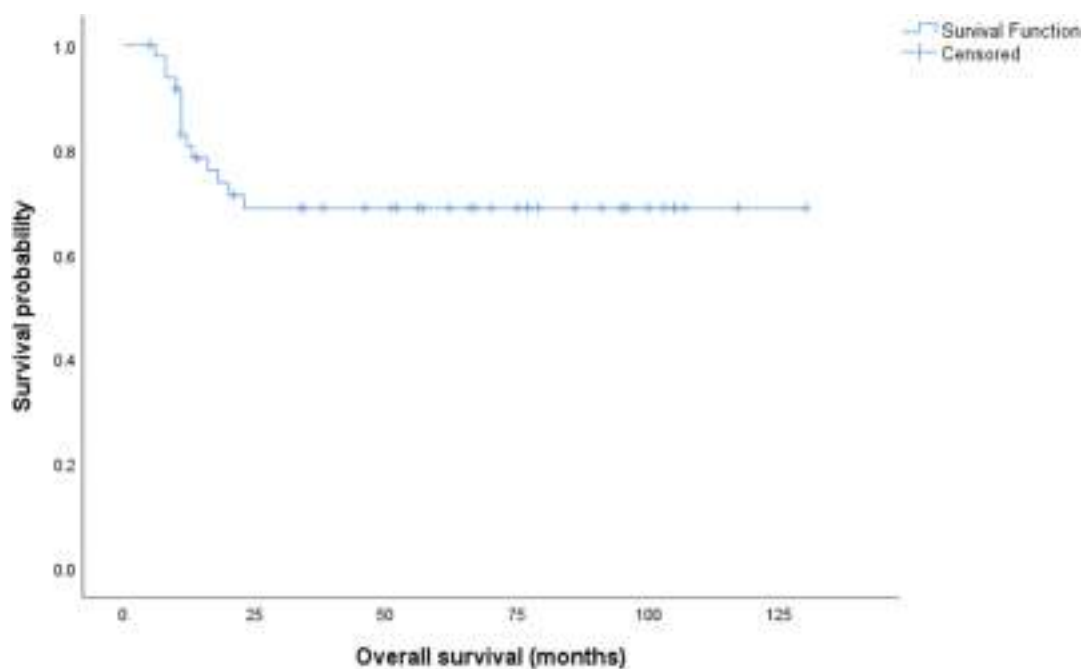


Figure 2 Overall survival in all patients.

After a median follow-up of 46 (range:5–130) months, 4-year PFS and OS were 60% and 71.4% respectively, [Figures 1](#) and [2](#). The median time to relapse or progression was 7.2 (range: 3.3 –14) months.

In univariate analysis, IPI >1 (mainly driven by advanced stage and ≥ 2 extranodal sites), [Figures 3](#) and [4](#), and B2M ≥ 2.5 correlated with PFS and OS, [Table 3](#). In multivariate analysis, IPI>1 significantly associated with PFS ($p=0.005$)

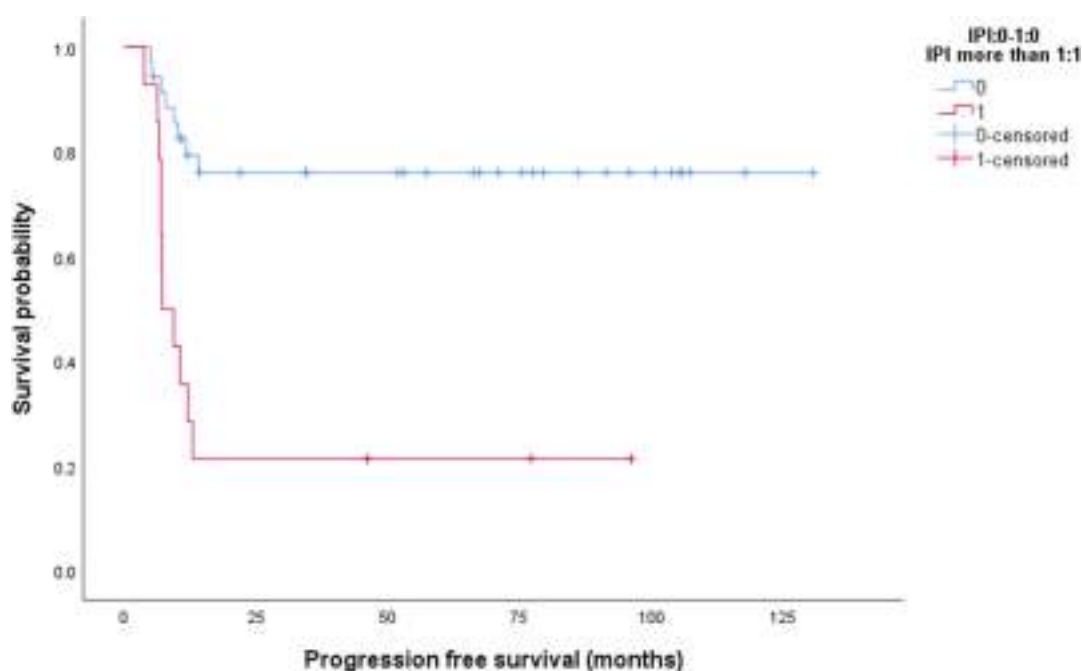


Figure 3 Progression free survival according to international prognostic index (IPI).

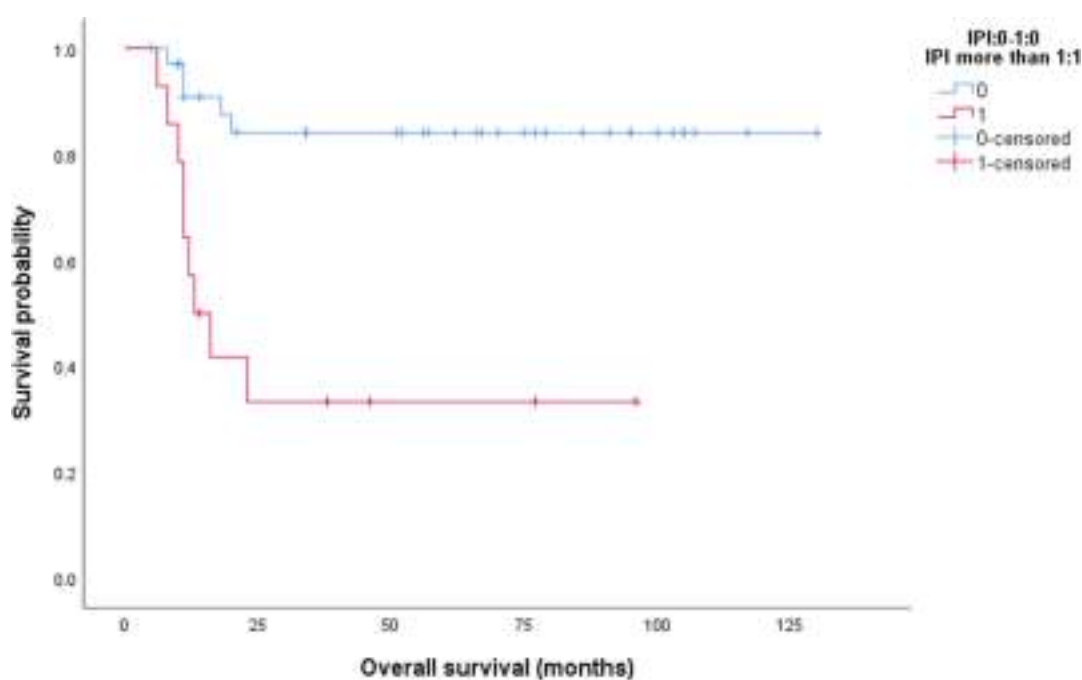


Figure 4 Overall survival according to international prognostic index (IPI).

and OS ($p=0.019$). In contrast, gender, the presence of B-symptoms, high ESR, and the presence of disease bulk had no statistically significant correlation with either endpoint, [Table 4](#).

Advanced stage disease ($p=0.009$), ≥ 2 extranodal sites ($p=0.005$) and IPI >1 ($p=0.009$) correlated with EOT response, [Table 5](#). The EOT response (CR vs no CR) correlated strongly with PFS ($p<0.001$) and OS ($p<0.001$), [Figure 5](#).

Table 3 Univariate Analysis for Progression Free Survival and Overall Survival

Variable	4-year PFS %	P value	4-year OS%	P-value
Gender				
Male	51.9%	0.196	65.3%	0.69
Female	69.7%		72.%	
Stage I–II	76%	<0.001	84.1%	<0.001
Stage III–IV	21.4%		33.3%	
Bulky disease ≥7cm	59.8%	0.13	65.8%	0.197
No bulky disease	100%		100%	
Bulky disease ≥10 cm	53.1%	0.193	64.4%	0.533
No bulky disease	70.5%		77%	
B-symptoms	53.3%	0.294	62.5%	0.257
No B symptoms	68.9%		78.2%	
<2 extranodal sites	71.4%	<0.001	80.7%	<0.001
2 or more extranodal sites	11.1%		20.2%	
ESR <50	66.3%	0.267	70.6%	0.335
ESR ≥50	64.7%		58.3%	
B-2 microglobulin <2.5	75%	0.034	78.1%	0.021
B2 microglobulin ≥2.5	45.5%		43.6%	
Normal LDH	83.3%	0.273	83.3%	0.489
High LDH	55.6%		65.7%	
IPI: 0–1	76%	<0.001	84.1%	<0.001
IPI >1	21.4%		33.3%	
Response to chemotherapy		<0.001		<0.001
Complete response	93.1%		92%	
Partial response	22.2%		55.6%	
Disease progression	0%		15%	
End of treatment response		<0.001		<0.001
Complete response	93.1%		92.6%	
No complete response	0%		26.9%	

Abbreviations: ESR, Erythrocyte sedimentation rate; LDH, Lactate dehydrogenase; IPI, international prognostic index.

Table 4 Multivariate Analysis

Progression Free Survival				
Variable	P value	HR	95% CI	
			Lower	Upper
B2 microglobulin	0.307	1.861	0.565	6.133
IPI	0.005	6.294	1.765	22.447
Overall survival				
B2 microglobulin	0.189	2.314	0.662	8.084
IPI	0.019	4.755	1.295	17.459

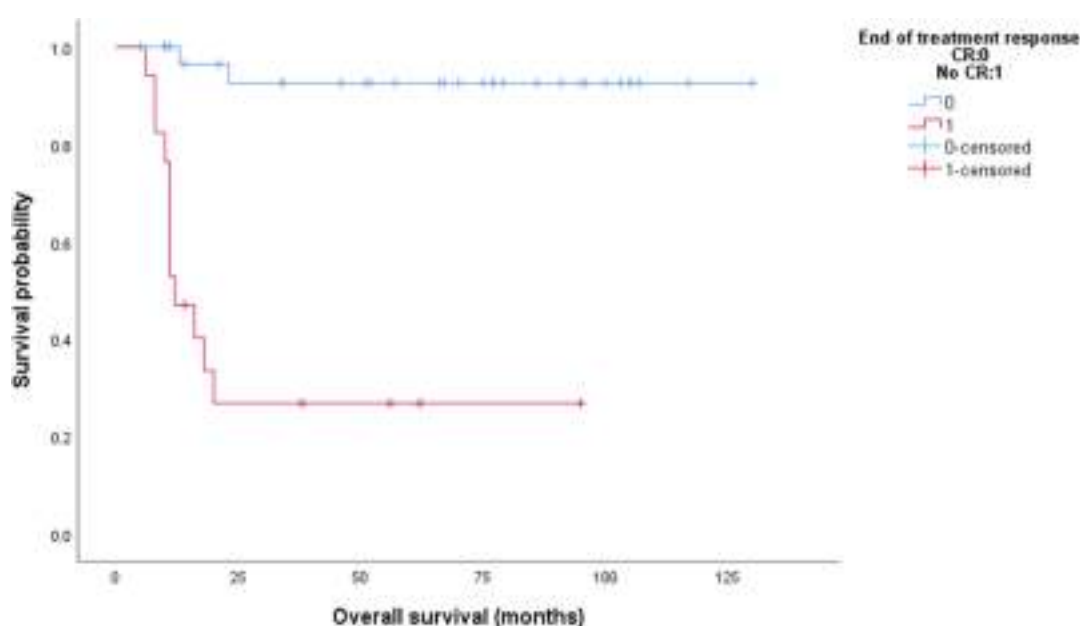
Abbreviation: IPI, international prognostic index.

Table 5 Correlation Between Different Pre-Treatment Variables and End-of Treatment Response

Variable	CR (%)	No CR (%)	P-value
Gender			
Male	50%	64.7%	0.378
Female	50%	35.3%	
Stage I–II	84.4%	47.1%	0.009
Stage III–IV	15.6%	52.9%	
Bulky disease ≥ 10 cm	59.4%	70.6%	0.541
No bulky disease	40.6%	29.4%	
B-symptoms	56.3%	42.9%	0.761
No B symptoms	43.7%	35.3%	
<2 extranodal sites	93.7%	58.8%	0.005
2 or more extranodal sites	6.3%	41.2%	
ESR <50	68.2%	42.9%	0.175
ESR ≥ 50	31.8%	47.1%	
B-2 microglobulin <2.5	78.7%	50%	0.13
B2 microglobulin ≥ 2.5	21.3%	50%	
Normal LDH	18.5%	6.7%	0.395
High LDH	81.5%	93.3%	
IPI: 0–1	84.4%	47.9%	0.009
IPI >1	15.6%	52.1%	

Abbreviations: ESR, Erythrocyte sedimentation rate; LDH, Lactate dehydrogenase; IPI, international prognostic index.

All patients with a relapsed or refractory disease (n=19) were treated with salvage chemotherapy, Table 1. 5 (26.3%) patients had a response; 3 went into CR (2 received pembrolizumab), and 2 achieved a PR. Patients with a response to salvage chemotherapy (n=5), received high dose chemotherapy and autologous stem cell transplant (ASCT), Table 2.

**Figure 5** Overall survival according to end of treatment response.

Abbreviation: CR, complete response.

Among the patients with a relapsed or refractory disease, 4 (21.5%) were alive with no disease on the last follow-up.

Discussion

There is no standard first line therapy for PMLBCL because of the lack of prospective randomized studies and no large registry reports published. The European Society of Medical Oncology (ESMO) guidelines concluded that RCHOP-14, RCHOP-21, DA-EPOCH-R, R-VACOP-B and V-MACOP-B are reasonable first-line options.²² RCHOP-21 with radiotherapy is the standard regimen recommended by the British Society of Haematology (BSH) guidelines and DA-EPOCH-R without radiotherapy can be used alternatively.²³ The National Comprehensive Cancer Network (NCCN) guidelines recommended either RCHOP or DA-EPOCH-R as first line therapy.²⁴

The most commonly used frontline regimens, RCHOP and DA-EPOCH-R, were compared in a multicenter retrospective study and showed higher rates of CR in DA-EPOCH-R (84.4% vs 69.6%) but no difference in long-term survival outcomes.²⁵ However, in another recent retrospective study, the use of DA-EPOCH-R was associated with improved PFS and OS in comparison with RCHOP.²⁶ Toxicity is an important consideration when selecting the optimal frontline treatment. DA-EPOCH-R is associated with more acute toxicities including neutropenia, stomatitis, cardiac complications, thrombosis, the need for admission and central venous access.²⁷

The PFS and OS rates in our study compare less favorably to Vassilakopoulos et al report, with 4-year PFS of 59% vs 80%, and OS of 71.4% vs 89%.²⁸ This can be explained by the inclusion of more patients with advanced stage disease in our study (21.4% vs 12%) and the fact that 18.4% of our patients had ≥ 2 extranodal sites, which was not reported by Vassilakopoulos et al, as the age-adjusted IPI was used to stratify the patients rather than the original IPI used in our study. This group had significantly worse outcomes (4-year PFS of 11% and OS of 22%). However, our results confirmed the reasonable survival outcomes of patients with low IPI (0–1) previously published in the MinT trial subgroup study.¹⁵

The results of our study highlight important practical points. First, patients with advanced stage disease or ≥ 2 extranodal sites had unsatisfactory outcomes when RCHOP was used in the frontline in accordance with the results of a recent study from British Columbia.²⁹ The use of DA-EPOCH-R in this high-risk group compares more favorably to our results (PFS of 60% vs 21.4%).²⁷ Although there are no prospective randomized studies, the use of more intensified approaches may be reasonable to improve long-term outcomes. On the other hand, for patients with limited-stage disease, RCHOP can accomplish the job.

Second, the median time to relapse or progression in our study was 7 months and none of our patients relapsed after 14 months. This observation was previously reported,³⁰ and may help to optimize patient's follow-up plan.

Third, the response to salvage chemotherapy in our study (26.3%), was also reported in other studies,^{31,32} indicating that refractory or relapsed PMLBCL, compared to DLBCL, is less likely to respond to salvage chemotherapy. Recently, the introduction of CD19 specific chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of relapsed DLBCL including PMLBCL. The results of two Phase 3 trials, the ZUMA-7 and the TRANSFORM trails, demonstrated a better event free survival and OS of CAR T cell therapy (axicabtagene ciloleucel in ZUMA-7 and Lisocabtagene maraleucel in TRANSFORM) relative to salvage chemotherapy and ASCT in patients with primary refractory or early first relapse (<12 months) large B cell lymphoma including PMLBCL.^{33,34}

In line with frequent expression of programmed cell death-1 (PD-1) and CD30 positivity in PMLBCL, CheckMate 436, KEYNOTE-170 and KEYNOTE-013 trials showed high efficacy of immune check point inhibitors as monotherapy or in combination with brentuximab vedotin in relapsed and refractory PMLBCL.^{35,36} Two of our patients were treated with pembrolizumab as a third line salvage went into CR and received high dose chemotherapy and ASCT. Both patients are still alive and disease free at the last encounter. The use of these agents may be considered in patients not eligible or relapsing after CAR T cell therapy.

The role of consolidative radiotherapy in patients treated with RCHOP is still an area of debate. Recent reports have shown that radiotherapy can be safely omitted in patients having a complete metabolic response on PET-CT scan.²⁶ Due to the small number of patients who achieved a CR and did not receive consolidative radiotherapy in our study ($n=8$), no solid conclusions can be made. The ongoing IELSG 37 phase 3 trial is investigating the role of consolidative radiotherapy after chemoimmunotherapy in PMLBCL would hopefully help to answer this critical question.³⁷

Our study has several limitations including the retrospective design, small number of patients and no comparator arm.

Conclusion

The use of frontline therapy with RCHOP in PMLBCL is suboptimal but may be reasonable in patients with low IPI score with good long-term survival outcomes. The use of more intensive approaches may be considered for patients with advanced stage disease. Patients with relapsed or refractory disease had a limited response to salvage chemotherapy and CART T cell therapy should be considered.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Cancer Incidence in Jordan. Available from: www.moh.gov.jo/c602eda7-0c36-49cd-bea1-3484e46c0b97. Accessed September 4, 2022.
2. Johnson PW, Davies AJ. Primary mediastinal B-cell lymphoma. *Hematology Am Soc Hematol Educ Program*. 2008;2008:349–358. doi:10.1182/asheducation-2008.1.349
3. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003;198(6):851–862. doi:10.1084/jem.20031074
4. Lazzarino M, Orlandi E, Paulli M, et al. Primary mediastinal B-cell lymphoma with sclerosis: an aggressive tumor with distinctive clinical and pathologic features. *J Clin Oncol*. 1993;11(12):2306–2313. doi:10.1200/JCO.1993.11.12.2306
5. Dabrowska-Iwanicka A, Walewski JA. Primary mediastinal large B-cell lymphoma. *Curr Hematol Malig Rep*. 2014;9(3):273–283. doi:10.1007/s11899-014-0219-0
6. Savage KJ, Monti S, Kutok JL, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood*. 2003;102(12):3871–3879. doi:10.1182/blood-2003-06-1841
7. Twa DD, Chan FC, Ben-Neriah S, et al. Genomic rearrangements involving programmed death ligands are recurrent in primary mediastinal large B-cell lymphoma. *Blood*. 2014;123(13):2062–2065. doi:10.1182/blood-2013-10-535443
8. Steidl C, Gascoyne RD. The molecular pathogenesis of primary mediastinal large B-cell lymphoma. *Blood*. 2011;118(10):2659–2669. doi:10.1182/blood-2011-05-326538
9. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the world health organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leukemia*. 2022;36(7):1720–1748. doi:10.1038/s41375-022-01620-2
10. Hamlin PA, Portlock CS, Straus DJ, et al. Primary mediastinal large B-cell lymphoma: optimal therapy and prognostic factor analysis in 141 consecutive patients treated at Memorial Sloan Kettering from 1980 to 1999. *Br J Haematol*. 2005;130(5):691–699. doi:10.1111/j.1365-2141.2005.05661.x
11. Todeschini G, Secchi S, Morra E, et al. Primary mediastinal large B-cell lymphoma (PMLBCL): long-term results from a retrospective multicentre Italian experience in 138 patients treated with CHOP or MACOP-B/VACOP-B. *Br J Cancer*. 2004;90(2):372–376. doi:10.1038/sj.bjc.6601460
12. Zinzani PL, Martelli M, Bertini M, et al. Induction chemotherapy strategies for primary mediastinal large B-cell lymphoma with sclerosis: a retrospective multinational study on 426 previously untreated patients. *Haematologica*. 2002;87(12):1258–1264.
13. Avigdor A, Sirotkin T, Kedmi M, et al. The impact of R-VACOP-B and interim FDG-PET/CT on outcome in primary mediastinal large B cell lymphoma. *Ann Hematol*. 2014;93(8):1297–1304. doi:10.1007/s00277-014-2043-y
14. Savage KJ, Al-Rajhi N, Voss N, et al. Favorable outcome of primary mediastinal large B-cell lymphoma in a single institution: the British Columbia experience. *Ann Oncol*. 2006;17(1):123–130. doi:10.1093/annonc/mdj030
15. Rieger M, Österborg A, Pettengell R, et al. Primary mediastinal B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: results of the Mabthera international trial group study. *Ann Oncol*. 2011;22(3):664–670. doi:10.1093/annonc/mdq418
16. Wilson WH, Grossbard ML, Pittaluga S, et al. Dose-adjusted EPOCH chemotherapy for untreated large B-cell lymphomas: a pharmacodynamic approach with high efficacy. *Blood*. 2002;99(8):2685–2693. doi:10.1182/blood.v99.8.2685
17. Dunleavy K, Pittaluga S, Maeda LS, et al. Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. *N Engl J Med*. 2013;368(15):1408–1416. doi:10.1056/NEJMoa1214561
18. Dunleavy K. Primary mediastinal B-cell lymphoma: biology and evolving therapeutic strategies. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):298–303. doi:10.1182/asheducation-2017.1.298
19. Moskowitz CH, Schöder H, Teruya-Feldstein J, et al. Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in Advanced-stage diffuse large B-Cell lymphoma. *J Clin Oncol*. 2010;28(11):1896–1903. doi:10.1200/JCO.2009.26.5942
20. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059–3068. doi:10.1200/JCO.2013.54.8800
21. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med*. 1993;329(14):987–994. doi:10.1056/NEJM199309303291402
22. Vitolo U, Seymour JF, Martelli M, et al. Extranodal diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2016;27(suppl 5):v91–v102. doi:10.1093/annonc/mdw175
23. Cwynarski K, Marzolini MAV, Barrington SF, et al. The management of primary mediastinal B-cell lymphoma: a British society for haematology good practice paper. *Br J Haematol*. 2019;185(3):402–409. doi:10.1111/bjh.15731
24. Zelenetz AD, Gordon LI, Chang JE, et al. NCCN guidelines® insights: b-cell lymphomas, version 5.2021. *J Natl Compr Canc Netw*. 2021;19(11):1218–1230. doi:10.6004/jncn.2021.0054
25. Shah NN, Szabo A, Huntington SF, et al. R-CHOP versus dose-adjusted R-EPOCH in frontline management of primary mediastinal B-cell lymphoma: a multi-centre analysis. *Br J Haematol*. 2018;180(4):534–544. doi:10.1111/bjh.15051
26. Zhou H, Xu-Monette ZY, Xiao L, et al. Prognostic factors, therapeutic approaches, and distinct immunobiologic features in patients with primary mediastinal large B-cell lymphoma on long-term follow-up. *Blood Cancer J*. 2020;10(5):49. doi:10.1038/s41408-020-0312-7

27. Morgenstern Y, Aumann S, Goldschmidt N, Gatt ME, Nachmias B, Horowitz NA. Dose-adjusted EPOCH-R is not superior to sequential R-CHOP/R-ICE as a frontline treatment for newly diagnosed primary mediastinal B-cell lymphoma: results of a bi-center retrospective study. *Cancer Med*. 2021;10(24):8866–8875. doi:10.1002/cam4.4387
28. Vassilakopoulos TP, Pangalis GA, Katsigiannis A, et al. Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone with or without radiotherapy in primary mediastinal large B-cell lymphoma: the emerging standard of care. *Oncologist*. 2012;17(2):239–249. doi:10.1634/theoncologist.2011-0275
29. Hayden AR, Tonseth P, Lee DG, et al. Outcome of primary mediastinal large B-cell lymphoma using R-CHOP: impact of a PET-adapted approach. *Blood*. 2020;136(24):2803–2811. doi:10.1182/blood.2019004296
30. Giulino-Roth L. How I treat primary mediastinal B-cell lymphoma. *Blood*. 2018;132(8):782–790. doi:10.1182/blood-2018-04-791566
31. Kuruvilla J, Pintilie M, Tsang R, Nagy T, Keating A, Crump M. Salvage chemotherapy and autologous stem cell transplantation are inferior for relapsed or refractory primary mediastinal large B-cell lymphoma compared with diffuse large B-cell lymphoma. *Leuk Lymphoma*. 2008;49(7):1329–1336. doi:10.1080/10428190802108870
32. Vardhana S, Hamlin PA, Yang J, et al. Outcomes of relapsed and refractory primary mediastinal (Thymic) large B cell lymphoma treated with second-line therapy and intent to transplant. *Biol Blood Marrow Transplant*. 2018;24(10):2133–2138. doi:10.1016/j.bbmt.2018.06.009
33. Locke FL, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. *N Engl J Med*. 2022;386(7):640–654. PMID: 34891224. doi:10.1056/NEJMoa2116133
34. Kamdar M, Solomon SR, Arnason J, et al. Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial. *Lancet*. 2022;399(10343):2294–2308. doi:10.1016/S0140-6736(22)
35. Armand P, Rodig S, Melnichenko V, et al. Pembrolizumab in relapsed or refractory primary mediastinal large B-cell lymphoma. *J Clin Oncol*. 2019;37(34):3291–3299. doi:10.1200/JCO.19.01389
36. Zinzani PL, Santoro A, Gritti G, et al. Nivolumab combined with brentuximab vedotin for relapsed/refractory primary mediastinal large B-cell lymphoma: efficacy and safety from the phase II checkMate 436 study. *J Clin Oncol*. 2019;37(33):3081–3089. doi:10.1200/JCO.19.01492
37. IELSG37. A randomized, open-label, multicentre, two-arm phase III comparative study assessing the role of involved mediastinal radiotherapy after rituximab containing chemotherapy regimens to patients with newly diagnosed primary mediastinal large B-cell lymphoma (PMLBCL). Available from: <https://clinicaltrials.gov/ct2/show/NCT01599559>. Accessed December 12, 2022.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Effectiveness of COVID-19 Convalescent Plasma (CCP) During the Pandemic Era: A Literature Review

Usha Rani Kandula, Techane Sisay Tuji, Dinkinesh Begna Gudeta, Kassech Leta Bulbula, Anwar Abdulwahed Mohammad, Ketema Diriba Wari & Ahmad Abbas

To cite this article: Usha Rani Kandula, Techane Sisay Tuji, Dinkinesh Begna Gudeta, Kassech Leta Bulbula, Anwar Abdulwahed Mohammad, Ketema Diriba Wari & Ahmad Abbas (2023) Effectiveness of COVID-19 Convalescent Plasma (CCP) During the Pandemic Era: A Literature Review, Journal of Blood Medicine, , 159-187, DOI: [10.2147/JBM.S397722](https://doi.org/10.2147/JBM.S397722)

To link to this article: <https://doi.org/10.2147/JBM.S397722>



© 2023 Kandula et al.



Published online: 22 Feb 2023.



Submit your article to this journal



Article views: 360



View related articles



View Crossmark data



Citing articles: 5 View citing articles

Effectiveness of COVID-19 Convalescent Plasma (CCP) During the Pandemic Era: A Literature Review

Usha Rani Kandula , Techane Sisay Tuji, Dinkinesh Begna Gudeta, Kassech Leta Bulbula, Anwar Abdulwahed Mohammad, Ketema Diriba Wari, Ahmad Abbas

Department of Nursing, College of Health Sciences, Arsi University, Asella, Ethiopia

Correspondence: Usha Rani Kandula, Department of Nursing, College of Health Sciences, Arsi University, Oromia Region, P.O. Box-396, Asella, Ethiopia, Tel +251-939052408, Email usharani2020@arsiun.edu.et

Abstract: Worldwide pandemic with coronavirus disease-2019 (COVID-19) was caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). As November 2, 2022, World Health Organization (WHO) received 628,035,553 reported incidents on COVID-19, with 6,572,800 mortalities and, with a total 12,850,970,971 vaccine doses have been delivered as of October 31, 2022. The infection can cause mild or self-limiting symptoms of pulmonary and severe infections or death may be caused by SARS-CoV-2 infection. Simultaneously, antivirals, corticosteroids, immunological treatments, antibiotics, and anticoagulants have been proposed as potential medicines to cure COVID-19 affected patients. Among these initial treatments, COVID-19 convalescent plasma (CCP), which was retrieved from COVID-19 recovered patients to be used as passive immune therapy, in which antibodies from cured patients were given to infected patients to prevent illness. Such treatment has yielded the best results in earlier with preventative or early stages of illness. Convalescent plasma (CP) is the first treatment available when infectious disease initially appears, although few randomized controlled trials (RCTs) were conducted to evaluate its effectiveness. The historical record suggests with potential benefit for other respiratory infections, as coronaviruses like Severe Acute Respiratory Syndrome-CoV-I (SARS-CoV-I) and Middle Eastern Respiratory Syndrome (MERS), though the analysis of such research is constrained by some non-randomized experiments (NREs). Rigorous studies on CP are made more demanding by the following with the immediacy of the epidemics, CP use may restrict the ability to utilize it for clinical testing, non-homogenous nature of product, highly decentralized manufacturing process; constraints with capacity to measure biologic function, ultimate availability of substitute therapies, as antivirals, purified immune globulins, or monoclonal antibodies. Though, it is still not clear how effectively CCP works among hospitalized COVID-19 patients. The current review tries to focus on its efficiency and usage in clinical scenarios and identifying existing benefits of implementation during pandemic or how it may assist with future pandemic preventions.

Keywords: convalescent plasma, COVID-19 convalescent plasma, effectiveness, CCP, COVID-19, coronavirus disease-2019, SARS-CoV-2, severe acute respiratory distress syndrome-coronavirus-2, pandemic era, literature review

Introduction

In recent years, coronavirus infections have been the source of epidemics, and the concern was raised about SARS (Severe Acute Respiratory Syndrome) and MERS in 2002 and 2012, respectively. The SARS-CoV-2, a current recruit among coronavirus family with the most recent outbreak and designated with COVID-19.¹ The worldwide epidemic has been reported due to COVID-19² and, the WHO proclaimed worldwide pandemic immediately after its development and subsequent spread among human population during 2019. Around 4.4 million people have perished from virus affects, and infected with a minimum of 215 million.³ At that movement, WHO stated on March 11, 2020, the spread of COVID-19 may pose significant and ongoing problems to the entire globe.²

Patients with COVID-19 are usually undiagnosed or exhibit mild respiratory concerns,⁴ moreover, some patients appear with a variety of symptoms, ranging from mild or flu-symptomatology (81% of cases) to acute (14% of cases) and

with severe (5%) clinical manifestations.⁵ In addition to affecting people's daily lives, the SARS-CoV-2 infection also led to organ damage. An evidence reported that the SARS-CoV-2 either directly or indirectly promotes inflammasomes and, by inducing pyroptosis, an inflammatory form of cell demise, and aggravating tissue injury with endothelial malfunction, vasodilation and, the inflammatory cytokines may promote tissue damage and, the patient may exhibit with several clinical manifestations,^{2,6} and multiple-organ failure may occur as a severe side effect of this inflammatory reaction.²

The Acute respiratory distress syndrome (ARDS), abnormal coagulation, multiple organ failure syndrome, or septic shock are some examples of infection processes other than direct viral infection that can cause severe effects in SARS-CoV-2. In some circumstances, adapted immunity is suppressed, which forces the innate immune system to respond more vigorously, and produced more inflammatory compounds than it should, may delay the clearance of viruses, and increase the number of immune cells that are activated at inflammation sites.⁷ During pandemic demands, there is a need to move swiftly to offer therapeutic options in this circumstance. Considering advancements in vaccination and pharmaceutical research, notably medications using nanotechnology and naturally occurring bioactive constituents,⁸ and with particular coronavirus's genetic diversity and rapid evolution, there is presently no special, effective treatment to prevent or treat the disease.^{9,10}

People who are critically ill are more at risk of dying from an overactive immune system than from a viral infection alone. Significant increases in cytokines and other inflammatory biomarkers, such as interleukins, interferon, tumor necrosis factors, colony-stimulating factors, growth factors, ferritin, C-reactive proteins, and D-dimers, are present among majority of patients in the intensive care unit (ICU) with severe COVID-19.^{11,12} Infiltration of further immune cells, such as lymphocytes, macrophages, and dendritic cells, may ensue from this excessive and prolonged cytokine response, which may exponentially increase the inflammation.^{11,13} The patients with COVID-19 may experience illness exacerbation and mortality mostly because of this immune system imbalance, which causes a cytokine storm.¹⁴ To prevent the illness worsening and lower mortality among COVID-19 patients, treatment may include rapid control of the cytokine storm syndrome (CSS) in addition to supportive therapies.^{11,15}

For the movement, the sole option for new coronavirus pneumonia is general supportive care only,⁴ with this reasons, from the hospital patients died at a rate ranging from 11% to 15%. Moreover, the amount of knowledge accessible in literary texts and official sources is steadily growing, despite the reality that COVID-19 is extremely contagious and has a high death rate.² Since late 2019, COVID-19 has become a concern for public health all around the world, and there has been a significant amount of debate and research towards finding a solution. Although, few strategies have proven to be effective, while others have failed.^{16,17} In addition to that, almost two years into the COVID-19 epidemic, we still have a limited pharmacological arsenal to prevent or cure the disease's devastating effects among affected population.^{18,19}

In progress towards the introduction of novel variations emphasizes the need for surveillance systems with update vaccination programs and treatment methods.²⁰ These variances are particularly worrisome because many regions of the world do not vaccinate their inhabitants due to a lack of infrastructure for mass manufacture and distribution, affordability and, with appropriate provisions.²¹ There are still many active infections with SARS-CoV-2 infectivity, transmission, and fatality are altering even though the percentage of people globally who are protected against COVID-19 is expanding as a consequence of the availability of various vaccinations.¹⁴ In this scenario, immunotherapies, cytokine inhibitors, monoclonal antibodies, and anti-inflammatory medications are instances of potentially effective therapy alternatives for COVID-19 prevention,^{22,23} and by removing inflammatory cytokines and infective markers, therapeutic plasma exchange may be a useful treatment for CSS.^{24,25}

Convalescent Plasma Therapy (CPT)

CPT is a passive immunization technique that has been employed for more than a couple of centuries as a reliable and efficient therapy for a wide range of infectious diseases, including COVID-19,²⁶ and it has been routinely employed in the treatment of hospitalized COVID-19 patients.^{27,28} CCP was a common form of passive immunization during the initial stages of pandemic, with the risk of antibody-dependent acceleration of viral illness even if the preventative vaccine and monoclonal antibody treatment had been quickly developed and used³ (Figure 1).

The United States Food and Drug Administration (FDA) currently suggests antivirals like remdesivir and anti-inflammatory medications like corticosteroids for hospitalized patients with who require supplementary oxygen (O₂), as

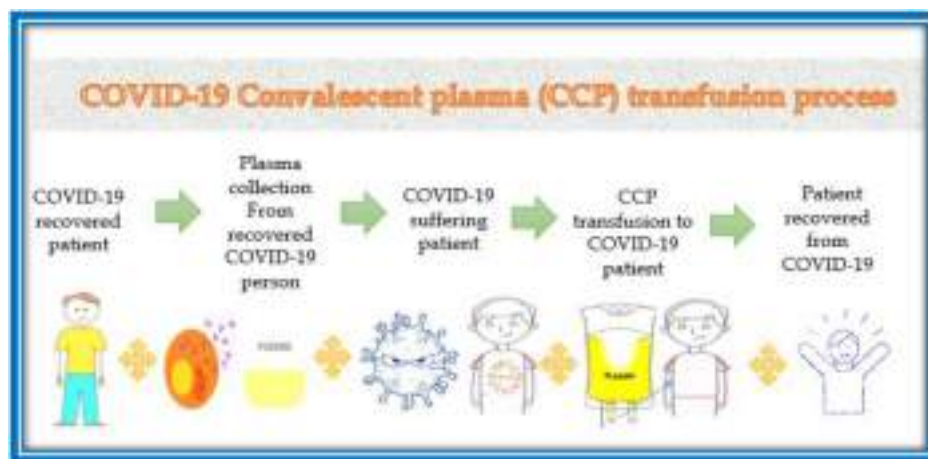


Figure 1 COVID-19 Convalescent plasma (CCP) transfusion process.

well as monoclonal antibodies targeted against SARS-CoV-2 spike protein for outpatients at high risk of disease development.²⁹ As everyone is aware, passive immunization has played an essential role in the treatment of infectious illnesses.³⁰ Soon as the commencement of COVID-19 epidemic, treatment options were predictably limited, forcing the reintroduction of a venerable method with passive immunization by transfusion of CP.³¹

Plasma transfusion from recuperating donors to acutely infected patients was one of the few treatment options available at the outset of the pandemic. In areas with limited resources, passive immunization employing CCP from formerly infected donors is still an effective therapeutic option. Despite a few positive results from CCP transfusion in patients with acute SARS-CoV-2 infection, the efficacy of this therapy still seems to be poorly or not thoroughly grasped.³² As before COVID-19 pandemic, clinical trials with CP were rare and produced contradictory results with range of research designs, patient demographics, infecting pathogens, timing of infusion delivery about the duration of the illness, dosage, and the controls used in previous research.^{33,34}

Moreover, High-titer CCP early delivery was shown to be safe and effective, with a lower risk of hospitalization and mortality compared with control groups.^{35,36} These combined findings have influenced clinical guidelines that advocate for the early transfusion of CCP.³⁷ The option of CCP is also available to hospitalized patients who are unable to take other available antiviral medicines due to safety concerns or contraindications, such as pregnant women, and patients with severe chronic kidney disease, or severe chronic cirrhosis of the liver.³⁸

During the 1918 Spanish flu pandemic,^{39,40} and subsequent severe viral diseases, including SARS, H₁N₁ influenza, MERS, and the Ebola virus, we later adopted CP infusion method as passive immunization.^{41–43} The pre-clinical animal models suggested that passive immunization therapeutics would be useful during the COVID-19 pandemic,^{44–46} and non-human primate studies discovered that passive transfer of immunoglobulins could confer therapeutic efficacy after viral exposure and fight infection in a dose-dependent manner.^{47,48} Additionally, case studies conducted early during pandemic demonstrated on positive outcomes for patients receiving CP from donors recovered through CCP transfusions.^{49–51}

As anti-SARS-CoV-2 neutralizing antibodies (NAb) are present in CCP and may be utilized as COVID-19 passive immunization in patients with high risk of severe illness and, also the plasma from recuperating patients may have additional immune-modulatory abilities when administered to COVID-19 patients.⁵² Additionally, the use of CCP from previously infected individuals to passively transfer anti-SARS-CoV-2 antibodies and potentially treat or prevent COVID-19 is currently being investigated. Furthermore, CCP is acquired from individuals who have fully recovered from illness and preferably includes high titer of viral NAb.⁵³

Reduced Plasma Cryoprecipitate (Cryosupernatant)

As recent research findings suggest on using fresh frozen cryoprecipitate-depleted plasma or plasma with reduced cryoprecipitate instead of apheresis plasma from recovered COVID-19 patients as a source of anti-SARS-CoV-2 antibodies.⁵⁴ Since Judith Pool's original proposal in 1964,⁵⁵ the vast majority of blood manufacturers have been able to produce cryoprecipitate supernatant from fast frozen plasma. The SARS-CoV-2 antibody-containing plasma is rapidly cooled to a temperature of -70°C or lower.

Treatment with CCP Among Other Viral Diseases

According to research that has been published, the CCP can be used to treat a variety of viral diseases, including influenza, Ebola virus, Spanish influenza, SARS, and MERS. The majority of patients observed positive effects from CCP transfusion without any challenges.^{56,57}

Ebola virus,^{56,57} the WHO has suggested the transfusion of CP or whole blood from patients who recovered from the Ebola virus as an empirical treatment for the Ebola outbreaks.⁵⁸

The Spanish flu,⁵⁷ as the studies that examined convalescent blood products to treat Spanish influenza-related pneumonia in hospitals as early as the 1918–1925 pandemic presented an assessment versus a control or comparison group. A meta-analysis performed in 2006, almost a century later, revealed a significant decrease in the total crude mortality rate, from 37% among controls to 16% among patients receiving CP.⁵⁹

H₁N₁,⁵⁷ a comparative study involving 99 patients, CP treatment was able to significantly lower respiratory tract viral load, serum cytokine response (interleukin-6, interleukin-19, and tumor necrosis factor- α), and mortality in context of pandemic influenza A (H₁N₁) 2009 virus infection. The probabilities of dying were reduced by 80%, making the decrease in mortality in that research study quite impressive.⁴²

SARS,⁵⁷ Following a comprehensive review and meta-analysis of 32 studies on severe influenza and the SARS coronavirus emphasized the consistent evidence for a decrease in mortality, particularly in cases of early administration of CP and hyperimmune immunoglobulin following symptom onset. The study results indicated a reduction in mortality odds of 75%,⁶⁰ supporting the significant reduction in mortality odds as per the investigation findings.⁶⁰

MERS,⁵⁷ the management of MERS with CP therapy planned protocol for people with illness was established during 2015. In accordance with protocol, individuals who had an anti-MERS coronavirus indirect fluorescent antibody titer of 1:160 or higher would be evaluated to identify, if they were eligible to donate plasma in accordance with standard donation guidelines and, provided that they had no signs of active MERS infection in the form of clinical or laboratory findings.⁶¹

The CP had also been used to treat measles,⁶² poliomyelitis,⁶³ and mumps,⁶⁴ during the first half of 20th century (Figure 2).

History of CP

Dr Emil von Behring introduced passive immunization as a revolutionary treatment modality at the turn of 20th century, and when he established that the plasma from horses afflicted with diphtheria or tetanus could be applied to heal humans also. He was awarded with Nobel Prize for Medicine or Physiology in the year of 1901. This discovery opened the door for the treatment of infectious diseases with plasma from recovering patients, and CP has been used throughout history to treat a variety of infections, including poliomyelitis, Spanish influenza, Korean hemorrhagic fever, and more notably, Ebola virus and influenza virus (H₁N₁).⁶⁵

The Arbeitskreis Blut, a working party as blood of the “German federal ministry of health”, released a statement (S16) on the production and administration of CP in severe disease epidemics on October 20, 2015. During early 20th-century, occurrences of diphtheria as well as subsequent cases of SARS, H1N1, and Ebola were said to respond positively to this therapeutic intervention.⁶⁶ As the first therapeutic efficacy using plasma with anti-SARS-CoV-2 antibodies from convalescent donors was reported by Chinese doctors at the same time.^{49,50,67} In addition to this, more than 15 years ago, the first CP infusions were undertaken to treat coronavirus infections. Further to this, the first published studies during the 2004 and 2005 Asian SARS outbreaks showed that patients who received plasma had shorter hospital stays and fewer deaths than those who received standard care.^{41,68}

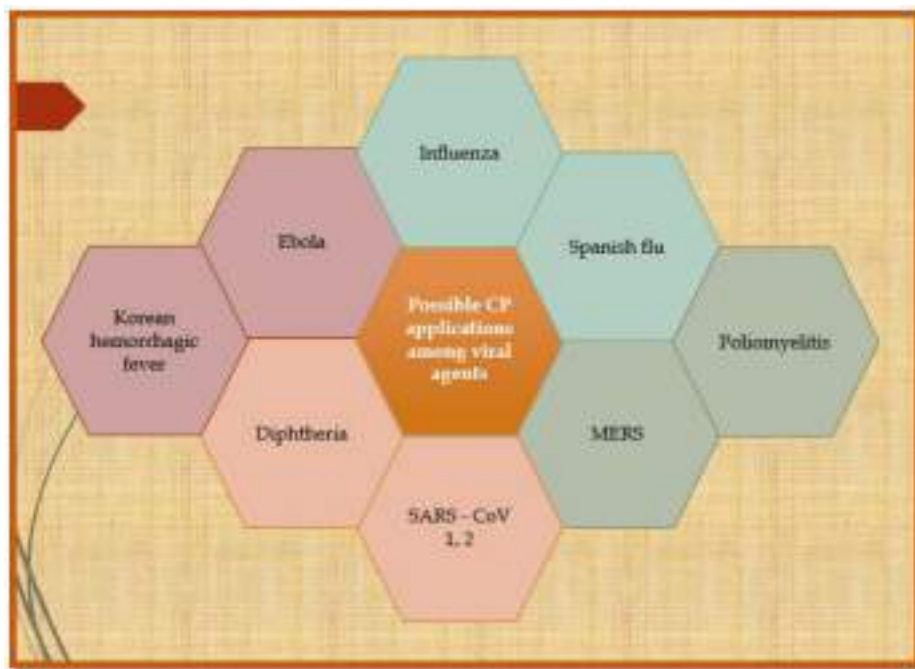


Figure 2 Possible convalescent plasma (CP) applications among viral agents.

According to the study, the first collection of CPT cases in SARS-CoV-2 patients was revealed by researchers in Asia at the beginning of 2020. As per the, first such trial, clinical and biological markers in five critically ill patients were reported to be improved by plasma infusion. There was also report on rapid neutralization of viral load process.⁵⁰ Further to this, the research paper below had 19 patients who had similar outcomes.⁶⁹ However, the European Commission developed guidelines for acquiring, analyzing, processing, preserving, disseminating, and regulating the use of CP in light of these preliminary results and the dearth of a viable etiological therapy for COVID-19, and the early applications of CP arose as an alternate therapy with a favorable safety profile in the absence of competent treatment.⁷⁰

CP Collection (CPC)

As per the following guidelines provided by the regulatory organizations of the nation in which it is delivered, CCP must be processed and utilized as an investigational patient's blood. According to US, the FDA in charge of enforcing rule that only registered and authorized blood collection facilities may obtain blood products as strictly. The regulatory body is also in charge of establishing the criteria for donors to be eligible, which have been modified as per new information has emerged over the course of pandemic. As to direct newly recovered donors to blood facilities early in the epidemic, several strategies were employed, such as testing databases with automated referrals, conventional and social media, etc.⁷¹

Apheresis Technology (AT)

As per the requirement of higher output of plasma volumes per donation (approximately three to four units for a large donor) in comparison to whole blood collection, apheresis technology is broadly applied for collection, at least in high-income nations in order to treat complex diseases.⁷² A medical treatment known as apheresis involves the temporary removal of blood components using centrifugal force, structural and size variations between blood components, and surface forces in microchannels as the basis of separation.⁷³

CP Donor Criteria

Plasma apheresis was performed on convalescent patients who were at least 14 days post-symptom resolution and eligible for plasma donation,⁷⁴ as well as remaining protocols as per the regular blood donation and transfusion procedures.

CP Testing

Testing for blood-borne diseases, ABO type, and human leukocyte antigens (HLA) (in the context of parous women) is necessary in addition to anti-SARS-CoV-2 antibody testing. Throughout the pandemic, antibody levels for CCP qualification have been regularly revised. Additionally, inconvenient for high-throughput screening, formal neutralization assays are also challenging as for standardization. Instead, several tests have been proven to be effective, with a certain antibody level being shown to correspond to neutralization.⁷²

CP Storage

Plasma treatment may be advantageous among patients with COVID-19 who have low humoral immunity or receiving B-cell depletion therapy for autoimmune diseases, multiple sclerosis, rheumatoid arthritis, or hematologic malignancies.^{75,76} Indeed, the beneficial effects of passive immunization on the clinical trajectory have been demonstrated, for instance, in a patient with a humoral immunodeficiency.⁷⁷

Institutional blood banks frequently keep CCP inventories. Each ABO-compatible type should ideally have an adequate supply to meet clinical demands, because only 4% of the population donates blood, and as demand for several blood type demands, particularly Group AB (universal donor), have proven challenging to collect as required quantity. These donations are usually intended to support the urgent use of plasma for the control of bleeding disorders. As Group “A” plasma may be substituted during routine trauma resuscitation if approved by the institutional policy since Group “AB” plasma as in limited supply conditions.⁷⁸

The CCP is still under investigation, which may restrict its use in comparison to plasma, which is frequently used to alleviate coagulopathy and hemorrhage conditions. As an organizational policy concerning non-group plasma should be formulated when transfusions are administered, and the patients should be informed on the benefits and dangers, and also the documentation for transfusion permission should include with any necessary disclosures as per the institutional policies.³⁸

Accordingly, clinical demand of CCP was higher than supply at the start of the epidemic. Because of the collection's rapid growth, most sizing-up blood facilities established a standing inventory, along with for responding to demand increases, having a CCP inventory on hand is crucial (eg, due to emerging variants). Later, as per the need decreased, and collecting came to an end as a result of vaccination process, and decrease in COVID-19 incidence, and evidence of the futility of treating advanced sickness. Due to this, earlier CCP inventories were inadequately suited to more recent variants like delta and omicron.⁷⁸

This highlights the necessity of keeping a small supply of CCP on hand as well as the ability to find new donors, especially those who have recovered from an infection with a virus that is the same as the one that infected the intended recipient. It is logistically challenging, even though temporal and geographic matching are preferred. Despite poor matching, qualified CCPs (as described by the FDA) are still thought to be helpful.⁷¹

CP Transfusion Area

To protect patients, workers, and visitors in healthcare facilities from infection, providing clinical treatment to potentially infectious patients during a pandemic necessitates a special infrastructure. Numerous outpatient transfusion and infusion centers provide immunosuppressive or chemotherapeutic treatment to sensitive populations, and sharing space with individuals who are actively infected with SARS-CoV-2 poses a serious risk to these populations.³⁸

It was a common infection control tactic to separate treatment facilities from ordinary care facilities. The logistical complexity of this system affects medical supplies and patients equally. Repurposing existing infrastructure for transfusion, such as negative pressure chambers used for the treatment of patients with airborne infections (such as TB), is one possibility for developing a new outpatient CCP transfusion center.³⁸

CP Usage

Passive immunization with CP treatment became more common in patients with SARS-CoV-2 infection during COVID-19. To properly determine the use of this treatment in which patient groups at what dose and at what appropriate time interval, no controlled trial has been carried out. Studies have revealed that CP therapy is well tolerated, early treatment decisions can reduce mortality and ICU stay length, and it is not just a last-resort rescue strategy⁷⁹ (Table 1).

Table Evidences from Literature Report on the Efficacy of Convalescent Plasma Administration Among COVID-19 Patients

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Nada Amri et al ¹³⁶	Plasma selection in COVID-19 CPT: Blood endothelial-cell extracellular vesicles.		24 April-12 July 2020		45 plasma donors who recovered from COVID-19 infection				Regarding obtaining COVID-19 CP, it is crucial to take into consideration the age of the donor, the period since the donor's initial clinical symptom first emerged, and the intensity of those manifestations.
Meenu Bajpai et al From India ¹³⁷	Effectiveness and safety evaluation of CP versus FFP in patients with severe COVID-19	Open-labelled, Phase II; pilot RCT.		Liver and biliary sciences, in coordination with the Internal medicine department, Lok Nayak hospital at Maulana Azad Medical College.	29 COVID-19 patients.		In patients with severe COVID-19 infection, one group (n = 14) obtained CP in addition to regular medical services, whereas group 2 (n = 15) obtained random donor FFP as a comparison in addition to usual medical services.	- At day 7, 78.5% of CP patients and 93.3% of control patients had not attained SS (p = 0.258). - The respiratory rate, oxygen levels, and SOFA score all significantly improved in the CP group. - Both groups did not experience any negative side effects after undergoing plasma transfusion.	In patients with COVID-19, CP treatment is secure and might be helpful.
Naveen Bansal et al ¹³⁸	An innovative system of grading to identify which COVID-19 CPT individuals need to receive special attention: a conclusion from a hypothesis					- Based on this grading methodology, 2 groups of patients are created. - Group at low risk (LRG; score <5) - Group at high risk (HRG; score ≥5)	- Score 0-4: No intervention (Basic standard care and hospital management) - Score of ≥5: Early CP therapy, preferably before 72 hours	To destroy the SARS-CoV-2 virus and prevent the CS from emerging, HRG should begin CP therapy within 72 hours following a definitive diagnosis of COVID-19.	In a specific group of patients where it is most likely to be effective, CPT must be used with prudence.
Giovanni Belcarì et al ⁹⁹	Clinical and viral reaction to CP in a patient with COVID-19 pneumonia and CLL (CR)				Male patient, age 62	- Patient treated on - Rituximab plus bendamustine - LMWH, - Azithromycin and ciprofloxacin - Low-flow O₂ support	CP in a COVID-19 Pneumonia patient with CLL	The patient had a full clinical, radiological, and virological response following 6 infusions of 600 mL of HTPC from recovering and triple-vaccinated donors.	This outstanding case adds weight to the expanding body of evidence demonstrating that, when HT dosages are given, CP is successful in curing ICPS.

(Continued)

Table (Continued).

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Artur Belov et al ¹⁰⁴	Clinical utility is moderate based on the earlier infusion of COVID-19 CP with a high AbsT concentration as determined by a live viral neutralized assay (ORA)				23,118 total patients - Received 1 unit of CP were separated into 2 groups those who received HTCP (>250 50%, ID 50; n = 13,636) and those receiving LTCP (≤250 ID 50; n = 9482).			HTCP transfusions led to absolute improvements in average 7 and 28-day DR of 1.1% and 1.7%, respectively, when compared to non-intubated patients who administered LTCP.	CP has only been demonstrated to have a moderate clinical benefit, but should viral variations emerge in the future that cannot be neutralized by currently available therapies, CP may play a role.
Mickael Beraud et al ²⁷	CP for the Tx of COVID-19: Lessons learned and particular considerations for patients with IDs (Review)		March 1, 2020, and May 1, 2021.		37 articles; 261 identified publications report reliable controlled studies.	From the 261 retrieved articles, 37 papers detailing robust controlled investigations in the whole population of patients with COVID-19 and 9 papers on ICPs with COVID-19 were selected.		Only CP units with high NAbT should be infused in patients with low NAbT to better prepare for future epidemics/pandemics and to assess the possible benefits of CPT.	Even while NAbT-containing CP infusions remain a safe, widely available, and potentially helpful therapeutic option for forthcoming pandemics and epidemics, CCP infusions did not improve COs for COVID-19 patients.
Rungsun Bhurayanontachai et al ¹³²	The outcome of non-ABO-identical CPT in ARDS related to COVID-19: A CR. (CR)				45-year-old man		- Favipiravir, lopinavir, ritonavir, hydroxychloroquine, azithromycin and IV methylprednisolone, dexamethasone, remdesivir 400 mL of CPT.	After plasma transfusion was finished, neither hemolytic nor non-hemolytic transfusion responses were found.	The ARDS patient, who was resistant to the current medical standard of care, reacted dramatically to an ABO-incompatible CP infusion without experiencing any TRs.
Soykan Bicim et al ²⁴	CP for the preventive Tx of COVID-19 in a patient with RF and HL (CR)				22-year-old female patient	6 cycles of Adriamycin, Bleomycin, Vinblastine, and Dacarbazine Tx with the diagnosis of HL 2 years ago - Rituximab, Cisplatin, Cytarabine, Dexamethasone (Chemotherapy) - HD and RRT for 6 times.	- CCPT - RRT - Favipiravir, remdesivir, lopinavir-ritonavir, oseltamivir	CP can be utilized as an additive therapeutic agent in patients where there is no standard Tx available and the DR is high owing to the underlying condition.	In this regard, immune CPT is a promising therapeutic procedure.

Yeliz Bilir et al ⁷²	• A comparison of the effectiveness of early versus late coronavirus treatment in patients requiring ICU (ORA)	RS evaluation	• CP therapy between April and June 2020	Kartal Dr. Lütfi Kırdar City Hospital	• ICU admissions of 20 patients	• CP Administration.	• CPT results at early (G1) and late (G2) periods were compared.	• Along with the delay of CP administration day, the length of stay in the ICU grew.	
Natalie Bruiners et al ¹³⁹	• Beneficial CPT in a patient with COVID-19 revealed disease-resolution pathways by its biological correlates (CR)					Hydroxychloroquine, methotrexate, rituximab infusion, azithromycin	• 4-liter supplemental O₂ • Day 40: 400 mL of COVID-19 CCP from an unidentifiable donor • The patient was given 600 mL of her relative's CP on day 54.	• On day 54, the patient's O₂ saturation increased to the point where supplemental O₂ was no longer needed.	• Prognostic hints in severe COVID-19 cases may come from tracking specific innate immune cell subsets in peripheral blood.
P. Cacilhas et al. From Southern Brazil ⁸⁶	• Patients with COVID-19 receiving CPT; an NRCCS with concurrent control (ORA)	NRCCS	October 2020 and March 2021		• 12 patients were assigned to the EG, and the CG had 24 patients who were matched for CCI.			• Patients who had CP had lower inpatient mortality rates than those who did not (RR: 0.48; 95% CI: 0.29 to 0.79).	• There were no variations in the rates of ICU admission across the groups (RR = 0.80; 95% CI: 0.47 to 1.35).
Natasha M. Clark et al ¹	• IgG and IgA Abs against SARS-CoV-2 do not promote viral infection in COVID-19 CP (ORA)				• 90 CCP donors			• These results support the notion that the IgG and IgA isotypes found in CCP do not influence the ADA of infection.	• The results thus indicate both the curative use of currently available Abs-based therapies, including the continuing use of CCP transfusion procedures, and the use of diverse vaccine portals in a prophylactic way.
Rongjuan Dai et al ²	• The SR and MA of CPT's safety and efficacy in COVID-19 subjects (Review)			• Databases from the Cochrane Central Register of Controlling Trials, PubMed, Embase, Web of Science, and China National Knowledge Infrastructure.	• 13 RCTs with a total of 13,232 participants			• According to the study's findings, there was a reduced MR in the CP group and an SS difference between the DR of the 2 groups compared to the CG (RR: 0.70, 95% CI: 0.55, 0.89, Z = 2.92, P < 0.004 0.01).	• CPT can improve breath, ICs IL-6, and ferritin in COVID-19 patients while reducing mortality with no significant increase in side effects.

(Continued)

Table (Continued).

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Giustina De Silvestro et al From Italy ¹⁴⁰	SARS-CoV-2 patient outcomes following CPT: a year's worth of data from the Veneto area registry in Italy (ORA)				209 out of 1517 patients who received CCP at the 30-day follow-up had died (14%).		A maximum of 3 therapeutic CCP fractions with a NAb:TA of less than 2:1:160 were administered to patients over 3 to 5 days.	Overall, mortality among patients receiving CCP was significantly ($p < 0.001$) lower (14% vs 25%), particularly in the older patient group (23% vs 40%).	The results demonstrate that, in the absence of specific adverse effects, early CCP Tx in patients with severe COVID-19 may contribute to a satisfactory result, with reduced mortality.
Giuseppe Gullo et al ¹⁴¹	CR and literature review on the usage of CP in women who are pregnant and have ARDS related to COVID-19 (CR)				34-year-old multipara woman with 27 weeks and 4th day of gestation.		CP administration	After a total of 43 days, the patient was discharged in good general health, afebrile, and without any respiratory symptoms or signs.	According to the research, the patient's health has temporarily and only partially improved. Unquestionably, a multidisciplinary team of obstetricians, anesthesiologists, and neonatologists should take the time of delivery into consideration.
Li Duan et al ¹⁴²	Studying the effects of CP on Abs alterations and NAC in COVID-19 patients.				299 COVID-19 patients, 200 cases in the non-plasma transfusion group.			IgM and IgG Abs were considerably (1–3 times greater) in the PIG compared to the non-PIG.	CPT considerably raises the Abs content in serious and critical in patients.
Emma Diletta Stea et al ¹⁴³	CPT in a SARS-CoV-2-infected HUS patient (Case Presentation)		October 2020	Polyclinic of Bari	Female Caucasian, 52 years old		- HIP - CPAP - Enoxaparin, Dexamethasone, Azithromycin, Ceftriaxone, Daptomycin	Blood gas measurement after the second plasma administration showed an improvement in the PO_2/FiO_2 ratio.	Using the HIP as a sort of rescue therapy may be beneficial for patients with a history of HUS and mild to severe SARS-CoV-2 infection.
Seyit Ahmet Erol et al ¹⁴⁴	An assessment of the outcomes of CPT given to pregnant women who were found to be COVID-19 positive in a pandemic center: A PC study (ORA)	-PC Study	28 August 2020 and 12 October 2020	Ankara City Hospital's OBG Dept	36 pregnant moms –12 mild and 24 moderate-severe.	Comparison in respect of clinical characteristics, laboratory parameters, obstetric complications, and neonatal outcomes	High neutralizing titers of 200 mL of CP ($>1/80$).	The moderate-severe COVID-19 group's O_2 saturation levels were considerably lower both before and after the administration of CP ($p < 0.05$)	Some research results suggest that in COVID-19 pregnant women with mild to moderate CP, early CP therapy may be associated with improvements in laboratory and ventilatory indicators.

Ignacio Esteban et al ¹⁴⁵	RCTs using CCP: Post-trial follow-up (ORA)	RCTs			• Older adults (142 patients)		• Participants were randomly assigned to receive either 250 mL of CP (with an IgG titer > 1:1000 towards SARS-CoV-2 SP) or 250 mL of placebo (0.9% NS) within 72 hours of the onset of symptoms.	• The chance of ARI-free survival (Log rank testing, p-value=0.63) and the percentage of ARIs (CP=11/72 and Placebo=9/70, p-value=0.678) among groups were both equal.	• Long-term COs or the risk of ARIs is not increased by COVID-19 CP, making it a safe intervention.
Noemi F. Freise et al ¹⁴⁶	• Analysis of the HR SARS-CoV-2 LEOSS cohort of patients receiving CPT using matched pairs (ORA)	• RS analysis			• 55 COVID-19 patients who are in hospitals		• 220 patients who received or did not get CP from the LEOSS-infected patients underwent an MPA (1:4).	• The overall intra-hospital DR was 41.8% (23/55), while the ISARIC-4C- Score predicted an MR of 26% (Range 0.3–59.1%).	• The largest mortality was found after CP Tx during the critical phase. • The data, however, lend support to the idea that early CP administration can reduce mortality.
Aliieh Fazeli et al From Iran ¹⁴⁷	• Tx with early HTCP in individuals with moderate to severe COVID-19 (ORA)	• PC Study	Affected individuals who acquired CCP between November 21, 2020, and March 20, 2021	Iranian BTO	• 3097 people who have COVID-19 in mild to severe form			• The study's findings showed that early CCP therapy was more effective for COVID-19 individuals who had moderate disease and were being treated with high Abs titers.	• A strategy for selecting potential CCP donors who have strong anti-SARS-CoV-2 IgG antibody levels is provided by the current study.
Diego Fernandez-Lazaro et al ¹⁴⁸	• SR of CPT and therapeutic drug combinations for COVID-19 epidemics in the 20th century (SR)		Articles published up to January 2022	• Web of Science, Scielo, Cochrane library plus, Scopus, and Medline (PubMed)	• ~6 RTCs			• According to the results, CPT with a quantity between 200 and 500 mL and a single transfusion administered in 1 to 2 h lowered viral load, symptoms, the duration of infection, and death in hospitalized COVID-19 patients without having any significant adverse side effects compared to the CG.	• CP did affect COs and might be a therapy option, but more research is required
Veronica Fernandez-Sanchez et al ¹⁴⁹	• A randomized, double-blind, two-center trial employing CP to treat Covid-19 (ORA)	• A randomized controlled double-blind clinical study.	May 20 to December 10, 2020		• 39 participants exhibited COVID-19 phases that were moderate (II) or severe (III).		• Patients allocated to CP received 2 units of 300 mL IV with a NAbT of ≥1:32, or 256 U/mL, the first unit on day 1 and the second unit on day 3.	• The 21-day post-transfusion mortality was significantly reduced in the CP group compared to the CG (HR: 0.17 [95.0% CI 0.07–0.45, p < 0.001]).	• Compared to CG in the CP group, CP lowers mortality, proving its effectiveness and safety.

(Continued)

Table (Continued).

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Daniele Focosi et al ⁹⁵	Potential use of CP in immunocompromised and susceptible people to prevent and treat SARS-CoV-2 (Perspective)								CP is a safe and efficient Tx for elderly ICPs when given while NAbT is high.
Ezter Fodor et al ¹⁰⁰	Early infusion of CP improves the CO in chronic SARS-CoV-2 infection (ORA)	A prospective, open-label interventional study							The research revealed that CCP transfusion is a safe and beneficial adjuvant therapy for hospitalized COVID-19 patients, with improved outcomes expected if utilized as early as possible.
Massimo Franchini et al From Italy ⁵¹	Single-center experience with CP for COVID-19 patients in hospitals (ORA)	RS cohort study	April 2020 and April 2021				657 CCP units were administered to 405 consecutive COVID-19 patients.	The 28-day crude MR was 12.6% (51/405)	Early HTCCP administration was the first of its type in the western world with COVID-19 patients, and it was a safe and effective treatment for hospitalized patients.
Massimo Franchini et al ¹⁵²	The seronegative hospitalized patient population's variant of concern-matched COVID-19 CP utilization (ORA)		October 2021 and March 2022	Single Italian hospital	17,200 regular blood donors		A high NAbT CCP unit (titred against by the specific VOC impacting the recipient) was administered to 21 hospitalized patients with severe COVID-19 utilizing CCP from 32 vaccinated and recuperating normal blood donors.	Patients median age was 66 years (IQR 50–74 years) - A 28-day MR of 14.3% (3/21) was documented, with age, advanced disease stage, and late CCP infusion connected with a worse prognosis.	This practical experience also lends credence to the administration of CCP to seronegative COVID-19 hospitalized patients during the Delta and Omicron waves.
Arvind Gharbharan et al ¹⁵³	Effects of Tx of coronavirus disease 2019 with CP in B-CD patients (Brief report)				~25 B-CD patients		CP with high NAbT.	Implying the potential therapeutic efficacy of this therapy in this specific demographic, 21 (84%) of the patients recovered.	Abs-based therapy may be advantageous for B-CD patients.

Oren Gordon et al ¹⁵⁴	Pharmacokinetics of HT anti-SARS-CoV-2 human CP in HR children (ORA)	Prospectively enrollment	May 2020 and April 2021	Johns Hopkins Children's Center	• 14 HR children		• Registered prospectively for HT COVID-19 CP (>1:320 anti-spike IgG; Euroimmun)	• HT COVID-19 CP was administered to 14 HR children (median age, 7.5 years), 9 of whom received it within 5 days (range, 2–7 days) after the beginning of symptoms and 5 of whom received it within 4 days (range, 3–5 days) of SARS-CoV-2 exposure.	• Regardless of weight or age, CP transfused to HR children seems to be safe and has the observed Abs kinetics.
R. Jain et al From India ¹⁵⁴	• Analysis of SARS-CoV-2 seroprevalence among potential CP donors and their deferral patterns: Results from a tertiary care institution in western India (ORA)	Prospective study	August 2020 to December 2020	• The department of blood banks at the tertiary care referral center, Goa medical college in western India.	• 400 prospective CP donors			• IgG SARS-CoV-2 Abs seroprevalence was 87% in prospective CP donors.	• 13% of patients who recovered from SARS-CoV-2 infection did not produce IgG Abs.
Satoshi Kutsuna et al From Japan ¹⁵⁵	• The effectiveness of CPT for COVID-19 patients and viral kinetics investigation (Brief Report)	• Clinical study with the open-label.		The Japanese Red Cross Central Blood Institute			• 11 people with COVID-19 mild to severe illnesses received CP.	• In just 28 days, clinical improvement was observed in 10 patients (91%).	• In the 11 patients who got plasma therapy, there were no side effects associated with the Tx.
Johan Kurnianda et al From Indonesia ¹⁵⁶	• CP Tx for people with moderate to severe COVID-19: A non-randomized comparative study with historical control in an Indonesian referral hospital (ORA)	• A non-randomized comparative study	June – September 2020	Indonesia's Yogyakarta Dr. Sardjito Hospital	• 15 from mild to severe sufferers of COVID-19.		• Plasma having an anti-SARS-CoV-2 specific IgG titer of more than 1:320.	• 10 survivors were left after 5 receivers died unexpectedly (a survival rate of 66.7%). • Recipients had a reduced MR compared to the controls (33.3% vs 46.7%; OR 0.75; CI 95% 0.17–3.33).	• CO for CP showed improvements, and recipients had a significant decrease in ILMs.
Judith Leon et al ¹⁵⁷	• COVID-19 CP from healthy and vaccinated donors have distinct SARS-CoV-2 Abs (ORA)				• 25 recipients of CCP.		• 25 recipients of CCP had their plasma samples taken before and after the transfusion, and the levels of COVID-19 Abs were assessed.	• Patients who received 2 units had a higher chance of seroconversion than those who only received 1 unit.	• It is recommended to use CCP from donors who received the vaccine and have very strong Abs levels.
Ferenc Magyari et al From Hungary ¹⁵⁸	• Remdesivir combined with CPT given early is efficacious in treating COVID-19 pneumonia in B-CD patients with HMs (ORA)	An observational, single-center study	December 2020 and July 2021	Covid-19 epidemiology care center, University of Debrecen, Hungary	• 20 hematological patients with B-CD.		• Every patient got a minimum of a full course of remdesivir and a minimum of 1 unit of CP.	• Remdesivir and CP were given simultaneously, and the patients' time for O2 weaning after diagnosis, hospital stay, and PCR positivity all decreased significantly in comparison to those who obtained the drugs independently ($p = 0.017$, $p = 0.007$, and $p = 0.012$, correspondingly).	• The clinical remission from COVID-19 and the viral elimination of SARS-CoV-2 are improved by early AVs Tx in conjunction with PI.

(Continued)

Table (Continued).

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Maddalena Marconato et al From Switzerland ¹⁵⁹	SARS-CoV-2 clearance is aided by CP Abs in both those with and without endogenous Abs responses (ORA)	Phase I clinical trial with non-randomization, open-label.	April and December 2020	One trial site, University Hospital Zurich in Zurich, Switzerland			From the same ABO-compatible plasma donor, a total of 600 mL were given over 3 days as 200 mL plasma units.	Both a parametric survival model (AHR, 3.0; 95% CI, 1.1–8.1; $P = 0.026$) and Kaplan–Meier analysis ($P = 0.034$) demonstrated a significant relationship between Tx with highly neutralizing plasma and faster virus clearance.	The lack of adverse events due to transfusions and the low MR (3.3%) in this trial demonstrated the efficacy of CPT.
Marcial Delgado-Fernández et al From Spanish ¹²¹	An analysis of the literature and 3 consecutive cases of COVID-19 Tx with CP in patients with HIDs (Review)		March to mid-June 2021		3 patients		Administration of CP in doses from 200 to 800 mL.	Following transfusion, abs levels were either low or negative, indicating that they may have been used to neutralize SARS-CoV-2.	COVID-19 and HIPs should be treated with CCP.
Antonio Mastroianni et al From Italy ¹⁶⁰	Obstacles with CP infusion therapy during pregnancy case report (CR)		November 9th, 2020	Anunziata Hospital in Cosenza	Pregnant Somali of 31 years	Dexamethasone, ceftriaxone, and enoxaparin fluids, - IV Supplemental O₂ - NEWS	The first CP unit was transfused on the first day, and the 2nd unit was infused on the second day, in addition to getting standard care.	CPT may be useful in pregnancy if used extremely early.	Despite the need for the findings of more comprehensive randomized studies, the administration of CPT may be regarded as a significant Tx option that does not harm pregnant people.
Flordeluna Mesina et al ¹⁶¹	MC research on individuals with COVID-19 who were hospitalized examined the use of CPT together with the best available Tx.	A quasi-experimental (prospective analytical), and MC study			65 COVID-19 disease patients who got CP.		Over 1–2 hours, patients were administered 250 to 500 mL of plasma while being monitored for TRs.	The length of stay between individuals who received CP and those who did not was found to differ by SS ($p = 0.00$).	Its use is safe for all patients, irrespective of the severity and clinical profile.
Katarina Nystrom et al ¹⁶²	An analysis of a severe COVID-19 and HIs patient using targeted T-cell responses to manage CPT (CR)				A male with X-linked agammaglobulinemia.		- Ig replacement therapy, spirometry, O₂, paracetamol, cefotaxime, doxycycline, and LMWH - On 2 consecutive days, the patient received 300 mL of AB0-matched CCP without experiencing any transfusion-related side effects.	Clinical management for COVID-19 patients with HIs may benefit from the evaluation of SARS-CoV-2T-cell responses.	

Alexandru Noris Novacescu et al ¹⁶³	CP transfusions followed by TPE in seriously ill COVID-19 patients: A single center non-RCT (ORA)	Single-center non-RCT	August 8, 2020, and January 9, 2021	Emergency Clinical County Hospital at Dr. Teodor Andrei Municipal Hospital's ICU.	38 Caucasian patients		TPE was initiated during the first 24 hours after ICU admission, and then CP was administered right away.	In the TPE with CP group, 30-day survival was 47.37%, compared to 26.32% in the CG ($P = 0.002$)	According to the study, severe and critically ill patients may benefit from an early initiation to TPE accompanied by CP infusion among COVID-19 individuals.
Alexandru Noris Novacescu ¹⁶⁴	CR: Combining TPE with CP transfusion led to the patient's successful recovery from critical illness after COVID-19 (CR)		January 2021 in the emergency room.	Dr. Teodor Andrei Municipal Hospital	52-year-old male		The patient had a 500 mL ABO compatible (A-II Rh-positive) CP infusion while being watched closely.	The condition of the patient recovered over the following days, and mechanical breathing was discontinued after the super infection was managed.	Early initiation of TPE followed by the infusion of CP may minimize the likelihood of the disease's progression and, ultimately, the probability of negative consequences in patients with severe and critical COVID-19.
Efry Sofyan Noor et al ¹⁶⁵	Impact of CP administration on elevating blood IgG and IgM levels to avoid Covid-19-related fatalities in the ICU (ORA)	Quasi experimental design		Panglima Sebaya Hospital, Paser Regency, East Kalimantan	50 patients		Administration of CP to COVID-19 patients in ICU.	The results showed that the IgG level was $269, 18 \pm 217.341$ before Tx and $1648, 1 \pm 490.140$ following Tx ($p\text{-value} = 0.000$). Before treatment, the IgM level was $78, 06 \pm 53.757$, and after treatment, it was $274, 16 \pm 160.908$ ($p\text{-value} = 0.000$).	According to the study's findings, CP boosted IgG and IgM levels in the ICU, preventing COVID-19-related deaths.
Alessandra Oliva et al ¹⁶⁶	CP for hematological patients with significant B-CD lymphocytes and SARS-CoV-2 pneumonia after anti-CD20 therapy (ORA)	RS observational study	October 2020 to May 2021		Six COVID-19 patients with non-HL and a lack of B-cells were studied.		CP was administered in the context of pneumonia with respiratory failure despite normal Tx and comprised 3 infusions on alternate days - Remdesivir, corticosteroids, and LMWH.	CP was provided 51 days (range 9–120) following SARS-CoV-2 diagnosis, and all patients improved quickly, except one patient.	The results of the trial revealed a quick clinical improvement and a probable survival advantage in COVID-19 patients with HMs and B-CD who had recurrent pneumonia managed with CP transfusion as a result of anti-CD20 therapy.
EA Ostrovskaya ¹⁶⁶	CPT efficiency and safety in COVID-19 patients: An SR		June 7, 2021, to December 20, 2021	PubMed, MedRxiv, Cochrane Library	29 full-text articles		RS and prospective studies related to CP therapy	The criteria for effectiveness and timing for transfusion of the CP were considered.	In seronegative patients with COVID-19 during an early stage of the illness or the existence of the IDs state, CP administration is safe and appropriate.

(Continued)

Table (Continued).

Reference and Author with Country	Title and Research Type	Research Design	Study Period	Setting	Population /Sample Size	Study Instruments	Intervention	Findings	Conclusion
Mila B. Ortigoza et al ¹⁶⁷	COVID-19 CP effectiveness and safety in hospitalized patients: an RCT (ORA)	CCP trial that was randomized, double-blind, and placebo-controlled design	April 17, 2020, to March 15, 2021	21 hospitals in 7 locations in New York, Connecticut, Florida, Houston, Texas, Maryland, and Wisconsin.	941 participants	PRISMA checklist	- Out of a total of 941 patients, 473 obtained a placebo, and 468 administered CCP. - Following 24 hours of the randomization, 1 unit of CCP, or approximately 250 mL, was delivered at a rate of no more than 500 mL/h.	In early analyses, CCP appeared to benefit those who were recruited when the majority of patients got HTCCP and were not receiving remdesivir and corticosteroids on randomization.	Participants in CP may have recovered earlier in the pandemic when remdesivir and corticosteroids were not prescribed.
Chun Pan et al ¹⁶⁸	CPT's efficacy in the management of COVID-19 infection among critically ill patients (ORA)	A case-matched RS cohort study	1 January 2020 and 29 February 2020	19 hospitals in the Hubei province's Wuhan and Huangshi have been designated for COVID-19.	26 patients in the group receiving CPT and 78 in the CG.		Patients who underwent CPT were matched to a maximum of 3 COVID-19 patients who did not receive CPT based on age, gender, DM, HTN, HF, duration between the start of symptoms and hospital admission, RSR, LC, troponin, SOFA, glucocorticoids, and AV medicines.	- However, the CP group had a higher SARS-CoV-2 negative conversion rate for 60 days following admission (26.9 vs 65.4%, $p = 0.002$) than the CG. - CPT did not affect the 60-day mortality rate (HR = 1.44, 95% CI 0.82–2.51, $p = 0.20$).	Patients with severe and potentially fatal COVID-19 infection may benefit from CPT, which may raise SARS-CoV-2 negative conversion rates but not their 60-day MR.
Hyung Park et al From New York ¹⁶⁹	CP may be beneficial for hospitalized COVID-19 patients; development and validation of a TBI (ORA)	The COMPILE trial's data was used in the prognostic study.	April 2020 to March 2021		A total of 2287 patients		- On days 14 and 28 following randomization, the 11-point WHO ordinal COVID-19 clinical status scale and its two derivatives (ie, WHO scores of 7–10 indicating MV to death and WHO scores of 10 indicating death) were administered. - From day 14, the WHO 11-point ordinal scale served as the primary metric for creating the TBI.	For the day 14 ordinal WHO score, the posterior median CCP efficacy OR was 0.94 (95% CrI, 0.74–1.19).	-The CCP TBI is a simple instrument that may be used to guide Tx suggestions by assessing the relative advantage of CCP Tx for a certain patient who is hospitalized with COVID-19. - The TBI precision medicine approach might be highly beneficial in the scenario of a pandemic.
A. Pratama et al ¹⁶⁹	CPT efficiency and effectiveness in COVID-19 patients. (Thematic poster session)		August-2022	Wahidin Sudirohusodo Hospital	6 COVID-19 patients		Six COVID-19 patients received 200 mL of double-routine CPT.	- In comparison to patients receiving standard treatment, CPT did not affect clinical improvement or death. - Clinical terms, chest X-rays, and RT-PCR conversion were improved in COVID-19 patients with severe instances.	The research found that patients with COVID-19 responded well to CPT.
Jinlv Qin ¹⁷⁰	A SR and MA found that plasma exchange reduced mortality in COVID-19 patients (SR & MA)		August of 2020	Embase, Cochrane Library, and PubMed	343 patients altogether; 170 were designated as CG, and 173 as TPE groups.	PRISMA	TPE therapy for COVID-19 in controlled clinical studies was compared with a standard of care.	TPE had a significant influence on mortality (RR 0.41, 95% CI 0.24 to 0.69; $P = 0.0008$).	TPE significantly reduced mortality in hospitalized patients with mild to serious COVID-19.

Zahra Soleimani et al ¹⁷¹	Midterm pregnancy ARDS caused by COVID-19: Effective therapy with plasma transfusion and corticosteroids (Short report)		02 April 2020		30-year-old woman		- Enoxaparin, Lopinavir/ritonavir, Azithromycin, Methylprednisolone, Hydrocortisone -CPT	Patients without concurrent bacterial infection may have a better CO with early delivery of HIP during the peak of the viremia and prudent corticosteroid therapy to dampen the CS.	Pregnant women with ARDS caused by SARS-CoV-2 infection but no concomitant bacterial disease may benefit from cautious corticosteroid therapy in conjunction with CPT to minimize viremia and CS.
Cristina Sanz et al ¹⁷²	Early CPT with HT SARS-CoV-2 NABs is effective in hospitalized COVID-19 patients (ORA)	Observational, propensity score MCCC design.	August 2020 to February 2021		261 of the 1604 patients that were examined received CCP, most of them (82%) within 24 hours of admission.		Each patient received 2 sequential 250 mL units of ABO-compatible CCP from the same donor.	Following PS matching, CCP transfusion was associated with a significant reduction in 30-day mortality (OR: 0.94, 95% CI: 0.91–0.98; $p = 0.001$) that lasted for 60 days following COVID-19 diagnosis (OR: 0.95, 95% CI: 0.92–0.99; $p = 0.01$).	The CCP may still be beneficial for some COVID-19 patients, and they urge additional research before CCP is taken out of the COVID-19 Tx arsenal.
Sapha Shabeeb et al ¹⁷³	CPT efficiency in COVID-19 patients' HMs (Review)			Scopus, Web of Science, PubMed, Science Direct, etc	258 COVID-19 patients who were receiving CP therapy for HMs.			CPT may be associated with improved clinical outcomes, such as (i) a higher survival rate, (ii) increased SARS-CoV-2 clearance and the existence of detectable anti-SARS-CoV-2 Abs following CP transfusion, (iii) expedited hospital discharge, and (iv) improved health after a month of CP therapy.	Due to its safety and good effects on COs, CPT appears to be a useful supportive therapeutic strategy for COVID-19-infected individuals with HMs.
D.J. Sullivan et al ¹⁷⁴	Early OP CPT for COVID-19 (ORA)	Double-blind, RCT	June 3, 2020, through October 1, 2021		Randomization involved 1225 people in all, and transfusions were given to 1181 of them.		A total of 592 patients received 333 units of Covid-19 CP from distinct donors.	The main outcome was experienced by 17 of the 592 individuals (2.9%) who received CP and 37 of the 589 participants (6.3%) who got control plasma (ARR, 3.4% points; 95% CI, 1.0 to 5.8; $P=0.005$), resulting in an RRR of 54%.	CP administration within 9 days of the onset of symptoms in individuals with Covid-19, most of whom were unvaccinated, reduced the likelihood of illness development resulting in hospitalization.

Abbreviations: ORA, Original Research Article; CR, Case Report; COVID-19, Coronavirus disease-19; CCP, COVID-19 convalescent plasma; CCPT, COVID-19 convalescent plasma therapy; CPT, Convalescent Plasma Therapy; CP, Convalescent plasma; FFP, Fresh frozen plasma; RCT, Randomized Controlled Trial; Dept, Department; NRCCS, Non-Randomized Case-Control Study; n, Sample Size; SS, Statistical Significance; O2 Saturation, Oxygen saturation; SOFA score, Sequential organ failure assessment score; HRG, High risk group; LRG, Low risk group; CS, Cytokine Storm; CLL, Chronic lymphocytic leukemia; LMWH, Low molecular weight heparin; HT, High Titer; HTCP, High-titer convalescent plasma; LT, Low titer convalescent plasma; LTCP, Low Titer Convalescent Plasma; ID, Inhibitory Dilution; NAbT, Neutralizing Antibody Titers; IV, Intra Venous; ARDS, Acute Respiratory Distress Syndrome; RF, Renal Failure; HL, Hodgkin Lymphoma; HD, Hemodialysis; RRT, Renal replacement therapy; ICU, Intensive care unit; G, Group; RR, Relative risk; CI, Confidence Interval; IgG, Immunoglobulin G; IgA, Immunoglobulin A; SR, Systematic Review; MA, Meta Analysis; MC, Multi Center; RS, Retrospective; CG, Control Group; Abs, Antibodies; PiG, Plasma Infusion Group; HUS, Hemolytic Uremic Syndrome; PC, Prospective Cohort; OBG, Obstetrics and Gynecology; HIP, Hyper Immune Plasma; CPAP, Continuous Positive Airway Pressure; PO2, Partial pressure oxygen; FiO2, Fraction inspired oxygen; NS, Normal Saline; ARI, Acute Respiratory Infection; HR, High Risk; LEOSS, Lean European Open Survey SARS-CoV-2; DR, Death Rate; PRISMA, Preferred reporting items for systematic reviews and meta-analyses; ISARIC-4C, International severe acute respiratory infection consortium clinical characterization protocol; SciELO, Scientific Electronic Library Online; MR, Mortality Rate; BTO, Blood Transfusion Organization; VOC, variants of concern; IQR, interquartile range; COs, Clinical Outcomes; PCR, Polymerase chain reaction; HIPs, Humoral Immunodeficiency Patients; NEWS, National Early Warning Score; HIDS, Humoral Immunodeficiency; Rh, Rhesus factor; TPE, Therapeutic plasma exchange; HMs, Hematological malignancies; DM, Diabetes mellitus; HTN, Hypertension; LC, Lymphocyte Count; CCI, Charlson Comorbidity Index; WHO, World Health Organization; MV, Mechanical Ventilation; CrI, Credible Interval; TBI, Treatment Benefit Index; RT-PCR, Reverse transcription polymerase chain reaction; NABs, Neutralizing antibodies; MCCC, Matched Case Control Study; RRR, Relative Risk Reduction; PS, Propensity score; ARR, Absolute risk reduction; ICs, Inflammatory cytokines; IDs, immunodeficiency; TRs, Transfusion reactions; Tx, Treatment; EG, Experimental Group; ADA, Antibody-dependent acceleration; NAC, Nucleic Acid Clearance; SARS-CoV-2, Severe acute respiratory syndrome-corona virus-2; COVID-19, Coronavirus 19; RA, Randomly Assigned; SP, Spike Protein; MPA, Matched Pairs Analysis; B-CD, B-cell depletion; IMs, Inflammatory Markers; AVs, Antivirals; HF, Heart Failure; OR, Out Patient; DAWn, plasma- Donated Antibodies Working against COVID-19.



Figure 3 Protective mechanism of Convalescent plasma (CP).

Protective Mechanism of CP

Neutralizing Antibodies Had Antiviral Activity

Significantly, patients with severe disease who received COVID-19 had stronger neutralizing antibody responses against SARS-CoV-2,^{80,81} and SARS-CoV-2 is eliminated by CP, which acts as an antiviral agent.⁵⁰

The Function of the ABO Blood Group

The observation that people with the blood type “O” are less likely to get a coronavirus infection than those with blood groups other than “O” was later noticed in SARS-CoV-2 infected subjects.^{82,83}

Anti-Inflammatory and Immune-Modulating Qualities

Anti-spike IgG and IgG non-neutralizing antibodies present in CP may also contribute to improving recovery in COVID-19 patients through their neutralizing effects.⁵³

Anti-Thrombotic Capability

CP, which is quickly frozen within six hours of collection and contains all pro- and anticoagulant elements in a physiologically balanced ratio⁸⁴ (Figure 3).

Discussion

The treatment to halt the course of early COVID-19 is still elusive, and CCP was first studied as a therapy among COVID-19 in patients.⁸⁵ As CP treatment improved mortality in COVID-19 hospitalized patients,⁸⁶ in contrast, the findings of another study on CP administered to hospitalized patients were ineffective, perhaps the antibodies needed to be administered earlier in the course of illness.⁸⁷ These results differed from earlier research among hospitalized COVID-19 individuals with CP transfusions, and no randomized studies have shown a benefit for plasma in reducing mortality so far.^{88,89} Furthermore, the benefits were indicated by Libster et al on earlier plasma provision with high antibody titers. Hence, the investigators further examined the antibody titer of plasma provision, and the early infusion of high titer CP among COVID-19 infections can stop disease progression.⁸⁷

At this point during pandemic, it looks like the compromised host with COVID-19 who is unable to mount their own antibody responses to the vaccine or past infection may benefit the most from passive antibody treatment. In that case,

particularly in the absence of additional antiviral medications or progression despite normal therapy, high-titer CCP from a recently recovered donor is a viable option.⁹⁰ The findings for monoclonal antibodies are highest when activity for the infective variation is maintained, and some but not all trials have indicated benefit for CCP. However, the precise niche for CCP and other antibody therapies remains unknown today, and in large part because of most available evidence was generated in the absence of current standard prevention and care strategies, such as vaccines and antiviral agents such as nirmatrelvir-ritonavir.⁹¹

The PLACID investigation (a multicenter clinical study) from India demonstrated that, while the CCP was effective in neutralizing the SARS-CoV-2 virus, it did not lower patient mortality.⁸⁹ This conclusion might be explained by the fact that the most frequent cause of illness among COVID-19 individuals were SARS-CoV-2 virus-induced cytokine storm instead of the SARS-CoV-2 virus directly. The most likely cause of CCP therapy's ineffectiveness on patient mortality is CCP infusion in people who have already developed significant COVID-19 symptoms. Moreover, non-neutralizing antibodies in CP can promote viral entry into macrophages. The virus multiplies rapidly in macrophages, creating a pro-inflammatory microenvironment that perpetuates the cytokine storm.⁹²

Some RCT results have consistently demonstrated clear benefits only among outpatients managed within 5 days of the initial symptoms.^{87,93,94} Based on an optimistic case study, and an extensive case series,^{95,96} the FDA reauthorized CCP transfusion for immune-compromised patients on January 2022. A rising amount of data endorses the benefits of hybrid plasma, ie, CCP acquired from convalescent donors who have been vaccinated at least twice,⁹⁷ as well as the significance of dose in CCP management.⁹⁸ As per the findings demonstrated, among patients received 6 units of plasma from donors who were both sick and triple-vaccinated, and as a response, their anti-spike antibody values were exceedingly high.⁹⁹

However, the initial data from the Mayo-sponsored extended access program revealed a positive safety profile among study samples; and adverse reaction rates were similar to those of non-immune plasma transfusions,³⁶ and several observational studies, or NRSs, revealed beneficial results when compared to matched controls.^{100–103} As per the given limitations of available data as on August 2020 and the extent of outbreak and CCP usage under the expanded access protocol was escalated, and initial statistics on the potential efficacy of CCP were necessary to support the regulatory decision-making process.¹⁰⁴

Recently, a mechanism on forecasting the potential benefit of CCP based on patient attributes was also revealed, and which may assist in identifying individuals who will benefit most from the CCP infusion,¹⁰⁵ and as per the findings on 7 recently published RCTs, on treatment of CCP appears to be associated with improved outcomes, among 20 COVID-19 patients.¹⁰⁶ As per the data on CP use for MERS,^{107,108} was limited, and studies in a small number of SARS patients suggested that CP may improve clinical outcomes when administered early in the illness or in those with severe disease.^{41,109,110} However, CCP treatment was initiated early in pandemic as a short-term strategy of providing immediate passive immunization to susceptible people and managing the disease until an effective and targeted drug could be discovered.¹¹¹

Primarily, CCP was employed in a variety of nations since it was the only readily accessible therapy capable of avoiding SARS-CoV-2 cellular infection, inhibiting viral replication, and curing COVID-19.^{111,112} Fairly, CCP could be obtained swiftly as the number of patients recovering from the illness rose in high-income countries by exploiting established blood collection and transfusion infrastructures.¹¹¹ Due to extended issues with donor recruitment, blood collection, inability to purchase CCP, and the characterization of CCP units, CCP was utilized less often in poor and middle-income countries during the early phases of the epidemic.¹¹³

The only difference between CCP and ordinary plasma infusions was that CCP contained anti-SARS-CoV-2 antibodies,³⁷ and CCP was generally given to seriously or critically ill patients who were regularly in the ICU or under mechanical ventilation during the early phases of the epidemic.^{114–116} As few trials found that CCP may be beneficial when administered to patients at an earlier stage of illness,^{117,118} but these findings were not substantiated by more recent RCTs.^{119,120}

The CCP NAb may assist in restricting disease progression and activation of the inflammatory cascade leading to a cytokine storm in the early stages of the disease by blocking the viral entrance and intracellular replication.^{87,112} According to 2 controlled studies in this cohort,^{119,120} CCP treatment was shown to be related to considerably increased survival rates, while an uncontrolled case series revealed that CCP infusions resulted in clinical benefits.^{121,122} In

Accordance with a preliminary study, immune-compromised patients with COVID-19 in an early stage of sickness and no detectable anti-SARS-CoV-2 antibodies are potential candidates for CCP treatment, and patients with a high post-transfusion antibody titer have the highest chance of success.¹²³

Further to a recent evaluation, CCP with high titer NABs is a safe and effective treatment for immune-compromised patients.⁹⁵ The reported benefits of CCP in these patients may be explained by their lower risk of hyper-inflammation and cytokine storm as well as their higher risk of persistent SARS-CoV-2 infections, which may be treated with CCP infusions.^{96,123}

Despite the low level of evidence for CP's efficacy against other coronaviruses, the data indicated that CCP might be a potentially helpful treatment for COVID-19 patients,²⁷ and CCP was associated with raised antibody levels but not with better outcomes in 59 patients compared to 15 controls.¹²⁴ Another trial found that CCP infusions were well tolerated by the population. There were no reports related to transfusion reactions.²⁷ A recently published RCT recommended that CCP with high titer NABs levels in addition to high IgG levels be used if more studies evaluated its use in persons with low humoral immunity.¹²⁵ So far, few data from adequately powered, randomized, controlled studies on CP therapy are available. In addition to that, several recent RCTs have revealed that CP treatment does not affect outcomes in patients with severe COVID-19.^{88,89}

However, the current evidence, which is reinforced by the publication of Donated antibodies working against COVID-19 plasma and the clinical research of Sekine et al,¹²⁶ does not warrant the use of CP in the standard COVID-19 treatment. More additional evidenced data is expected to emerge soon to help decide if CP transfusion, administered within 72 hours of the onset of symptoms, and may benefit some subgroups of individuals with very specific clinical and biological aspects. Mainly, these findings emphasize the need for restricting the compassionate use of treatment, conducting well-designed RCTs, and restricting treatments to those that are evidence-based.

As of July 8, 2022, the COVID-19 pandemic had caused 554 million illnesses and 6.35 million deaths worldwide. While the majority of immune-competent patients who have been triple vaccinated do not acquire severe illness following SARS-CoV-2 infection, immune compromised patients, particularly those with B-cell depletion, do not react to vaccinations and are thus at higher risk of sequelae.¹²⁷

CP is a potentially useful therapy; nevertheless, the data on its effectiveness to date has not been well studied and consistent. There are currently no protocols in place for collecting and administering it during pandemics, and collecting enough CP with high titer NAB to treat a substantial number of patients and quickly and efficiently performing RCTs with low bias risks during a pandemic are major obstacles. Currently, researchers have a better grasp on how to prepare for next epidemic or pandemic, more than 1.5 years after the COVID-19 pandemic began. According to the findings, the CP is only beneficial if the antibody levels in the infused units are high and the etiology of the sickness is unknown enough to determine the optimal therapeutic strategies.²⁷

Additionally, it is necessary to develop a reliable procedure for selecting potential donors who have been cured of the ailment. As a result, consistent viral nucleic acid diagnostics and antibody assays should be established as quickly as possible for screening necessities, and only men or nulliparous women with no history of transfusions should be regarded as donors in the absence of testing, as they should be anti-HLA negative.¹¹³

The establishment of a CP donor registry might be beneficial in identifying possible donors for future donations. A frozen and ready-to-use CP plasma bank might also be built by collecting plasma from all potential donors once or twice, especially in the early stages of a pandemic. It is critical to determine if the virus may be transmitted by transfusion, and pathogen inactivation techniques should be investigated until this is proven, particularly if prophylactic CP infusion after a potential exposure is contemplated. Furthermore, in epidemics or pandemics, finding CP patients with low antibody levels is crucial for implementing a successful CP strategy.²⁷

Adoption and application of CP treatment in later stages of pandemics may vary by nation. Because of practical problems, the early stages of CP therapy may be more difficult and necessitate unique solutions in underdeveloped countries. Researchers believe that CP, in addition to standard therapy, might be utilized to treat severe cases of COVID-19 pneumonia or individuals with decreased antibodies response to SAR-CoV-2 in the first week of symptoms or during the viremia phase.¹¹³

At this point in the pandemic, it looks like the immune-compromised patients with COVID-19 hosts who are unable to mount their own antibody response to the vaccine or prior infection will gain the most from passive antibody treatment. In that scenario, particularly in the absence of additional antiviral therapies or progression despite normal therapy, high-titer CCP from a recently recovered donor is a feasible strategy. In that context, findings for monoclonal antibodies are highest when activity for the infecting variation is maintained, and some but not all trials have indicated benefit for CCP.

Given the limited therapy choices and historical precedence, the FDA approved CCP for use beginning in April 2020. Concurrently, several RCTs with diverse trial designs and research populations, such as preventive, early outpatient usage, and hospitalized patients with severe disease, were approved under investigational new drug applications. Simultaneously, many RCTs with varied trial designs and research groups, such as prophylaxis, early opioid usage, and hospitalized patients with severe illness, proceeded forward under investigational new drug applications. Several observational studies, or NRSs, revealed beneficial results when compared to matched controls.

The case findings are as follows: the strengths of the analysis on early administration of CCP with high titer antibody content by live viral neutralization assay are associated with modest clinical efficacy, providing greater precision concerning CCP biologic activity and the power to detect modest treatment effects in the overall heterogeneous hospitalized population, reflecting real-world use of CCP in the early pandemic.¹⁰⁴

The findings of studies in the hospitalized population, where effects may be seen only in subgroups, must be interpreted in light of the timing of CCP transfusion relative to the onset of the illness, the host immunological condition, and the neutralizing activity of the CCP transfused. The inquiry into how to most effectively use CCP and which patients may benefit the most from it is ongoing; RCTs, as well as an analysis of subgroups within RCTs, will remain crucial in identifying the potential role of CCP in COVID-19 treatment. The CCP may have an influence on COVID-19 treatment in vulnerable patients in the future if variations arise that are not reduced by existing known treatments, despite having just a little clinical effect in the current trial.

A meta-analysis discovered that CCP treatment, like CP infusions, had a clinically acceptable safety profile in COVID-19 patients. While the bulk of the 7 newly published RCTs confirms CCP's reassuring safety profile, the death rate was significantly lower or tended to be lower in all COVID-19 patients who took CCP compared to control patients. Individuals who got CCP had a longer hospital stay in several trials. Other studies found that CCP improved disease progression, hypoxic condition mitigation, WHO intensity score, respiratory specifications, rate and time to clinical benefit, need for mechanical ventilation, extubation rate, time for recovery from major illness, rate of mechanical ventilation and vasopressor assistance, rate of transition to mechanical ventilation, and clinical status in the overall study participants or specific subgroups of participants.

The most prevalent CCP transfusion-related issues in certain countries include allergic transfusion reactions, transfusion-related acute lung damage, and transfusion-associated circulatory overload, all of which are curable. Another potential risk of CCP infusions was antibody-dependent acceleration of infection, which is a process in which non-NAbs, commonly created after a previous infection with a different viral serotype, increase viral cellular entry, exacerbating symptoms.^{128,129} This possible danger has not been observed with CCP infusions. Even though CP transfusions may be a beneficial treatment option in critically ill patients with other ailments, no positive impact of CCP was discerned in patients with COVID-19 at a delayed disease stage who were at high risk of death due to hyperinflammation or health complications rather than the SARS-CoV-2 infection itself.^{87,112}

For any future use of CP in the context of an emerging infectious disease outbreak, well-defined patient scoring systems based on characteristics other than time from symptom start or hospital admission or ICU are essential. In addition to symptom persistence (though disease progression varies from patient to patient), standardized criteria should be based on viral pathophysiology, illness intensity (eg, even without mechanical ventilation), and number of days post-hospital admission (correlated to symptom severity).

Later in the disease course, antibody testing may be useful in identifying patients who have not yet generated sufficient antibody levels and may benefit from CP. Furthermore, in people with early infection, binding antibody signals may not accurately indicate N-Abs levels and should not be used as the sole justification for commencing CP infusions.^{125,130} Another option for characterizing sickness stages is to routinely use the WHO clinical progression scale.¹³¹

CCP may enhance clinical outcomes in some subpopulations of COVID-19 patients, but its use in the general population does not appear to be beneficial. Researchers recognized immune compromised patients (eg, organ transplant recipients or patients with primary or secondary immunodeficiencies, B-cell depletion, or malignancies) as a prospective target population that may benefit more from CCP treatment based on existing research, clinical experience, and the pathophysiology of COVID-19.^{96,123,132}

Potentially CCP-related reactions included anemia, urticarial, nausea, shortness of breath, irregular heartbeats, and tachycardia, as well as local injection site reactions (pain, chills, rash, redness, and itchy skin), intravenous cannula obstruction, transfusion-related acute pulmonary injury, transfusion-associated cardiovascular burdening, respiratory, allergic, febrile non-hemolytic, and hypotensive reactions, etc.

The study demonstrated how a critical acute respiratory distress syndrome patient who was refractory to current standard medical treatment responded dramatically to ABO-incompatible CP transfusion without any transfusion reactions. They further argued that CP transfusion in severe COVID-19 pneumonia is clinically helpful, especially in the less compatible ABO-blood group. However, this is simply an anecdotal report; a larger evaluation of CP transfusion in critically ill COVID-19 pneumonia patients is necessary before this can be suggested as standard treatment.¹³³

Studies believe that the clinical improvement in the patient's condition was caused by other medications administered in conjunction with CP. In patients who do not have a conventional treatment and have a high death rate due to the underlying condition, CP can be used as an additional therapeutic agent. Finally, when administered to asymptomatic and immune-compromised patients with good infection control practices as soon as they are diagnosed, this therapy may be more beneficial.²⁶

Complications and Limitations of CP Applications

Adverse Reactions

There have been reports of adverse reactions ranging from mild fever to allergic reactions to life-threatening bronchospasm, circulatory overload in patients with cardiorespiratory diseases, and renal impairment especially, among the elderly population.^{111,134}

Immunological Reactions

The plasma administration may result in serious allergic responses. As allergic reaction to components of donor plasma or serum can cause serum sickness and anaphylaxis, and also bronchospasm may be linked to these reactions.¹³⁵

Risk of Transfusion-Associated Infections

Administration of CP carries possibility of transferring possible pathogens, including SARS-CoV-2 itself as well as other pathogenic agents such as hepatitis B and C viruses, human immunodeficiency virus, and *Treponema pallidum*. This risk may present even though it is pretty uncommon, and thus necessary to screen for existence of these pathogens in order to reduce the risk of infections related to transfusions.¹³⁵

Risk of Reinfection

Administration of CP, or passive antibodies, may decrease the formation of specific antibodies against SARS-CoV-2 by inhibiting or impairing the recipient's humoral immune response (pathogen-specific antibodies). This could increase a person's susceptibility to SARS-CoV-2 reinfection.¹³⁵

Antibody-Dependent Enhancement

There is a remote possibility of antibody-dependent enhancement of the disease process. It is a process in which antibodies present in the donor's plasma may exacerbate disease by enhancing the entry of viruses into host cells and the multiplication of viruses.¹³⁵

Other Adverse Reactions

There is a tiny possibility that an antibody-dependent enhancement of illness process may occur. As by facilitating virus entry into host cells and virus replication, antibodies found in donor plasma may aggravate illness.¹³⁵

One of the patients who underwent CP therapy reportedly developed an ephemeral facial red spot,⁴⁹ and there have also been reports on phlebitis and widespread jaundice in certain patients.

Other limiting factors may be a lack of neutralizing antibodies in patient plasma, large infusion volumes, and the time of infusion administration, waning of plasma antibodies, bridging the gap between COVID-19 positive and recovered cases, basic administrative and logistical barriers, and also donor eligibility criteria.

Conclusion

The stability and high potential efficiency of CPT as a COVID-19 therapeutic approach CP treatment can reduce mortality in COVID-19 while enhancing respiration, inflammatory cytokines, interleukin-6, and ferritin with no rise in adverse effects. For immune compromised patients who do not react to vaccinations, the COVID-19 burden remains unchanged. Additionally, CCP therapy is being utilized as an experimental treatment as early as January 2020, and with the developing understanding of COVID-19, CCP treatment has emerged as an essential experimental therapeutic being employed in the management of the disease. Moreover, CP treatment is risk-free and may help COVID-19 patients.

Furthermore, the multiple trials have demonstrated that CCP infusions are ineffective in treating COVID-19 patients, the issue of whether plasma from vaccinated individuals could be useful remains unanswered. Along this, it was recently demonstrated that, while immunization reduced in vitro neutralization capacity against specific variations, vaccinated people preserved neutralization capability against the majority of developing variants. As a result, gathering CCP from people who have been vaccinated and recovered from the first illness is important.

The storage of plasma acquired after vaccination may have a role in the creation of a more aggressive variant or during the present vaccination gap in some countries, assisting in better preparedness for the next wave of diseases. However, CP infusions do not appear to be effective in critically ill patients for some viral infections when the life-threatening symptoms are not the direct result of viral cellular damage, such as COVID-19, but may enhance clinical outcomes in specific subpopulations.

In addition to this, the COVID-19 needs more investigation to determine whether CCP infusions may be beneficial in the early stages of immune-compromised disease. Focusing to, the CP treatment may stop infections in future pandemics or epidemics and should not be confined to seriously ill individuals but should be delivered at the early stages of the disease to all patients or certain subpopulations of vulnerable persons. Interestingly, in an ideal world, a standardized protocol for RCTs studying CP safety and efficacy would be devised, made available, and ready for use internationally.

Data Sharing Statement

The study used data available within the manuscript.

Acknowledgment

Arsi University, College of Health Sciences, Ethiopia

Author Contributions

All authors made a significant contribution to the work reported, whether that was in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; they took part in drafting, revising, or critically reviewing the article; they gave final approval of the version to be published; they agreed on the journal to which the article had been submitted; and they agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Dhama K, Khan S, Tiwari R, et al. Coronavirus Disease 2019-COVID-19. *Clin Microbiol Rev*. 2020;33:e00028–20. doi:10.1128/CMR.00028-20
2. Dai R, Hu M, Tang H, Peng Z, Yan C, Emran TB. Efficacy and safety of convalescence plasma therapy in COVID-19 patients: a systematic review and meta-analysis. *Evid Based Complement Alternat Med*. 2022;2022:e7670817. doi:10.1155/2022/7670817
3. Clark NM, Janaka SK, Hartman W, et al. Anti-SARS-CoV-2 IgG and IgA antibodies in COVID-19 convalescent plasma do not enhance viral infection. *PLoS One*. 2022;17(3):e0257930. doi:10.1371/journal.pone.0257930
4. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus Disease 2019 (COVID-19): a Review. *JAMA*. 2020;324(8):782–793. doi:10.1001/jama.2020.12839
5. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA*. 2020;323(13):1239–1242. doi:10.1001/jama.2020.2648
6. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: a systematic review and meta-analysis. *Rev Med Virol*. 2020;30(6):1–9. doi:10.1002/rmv.2141
7. Chen R, Lan Z, Ye J, et al. Cytokine storm: the primary determinant for the pathophysiological evolution of COVID-19 deterioration. *Front Immunol*. 2021;12:589095.
8. Rahman M, Islam M, Shohag S, et al. Multifaceted role of natural sources for COVID-19 pandemic as marine drugs. *Environ Sci Pollut Res*. 2022;29:46527–46550. doi:10.1007/s11356-022-20328-5
9. Phan T. Genetic diversity and evolution of SARS-CoV-2. *Infect Genet Evol*. 2020;81:104260.
10. Islam F, Dhawan M, Nafady MH, et al. Understanding the omicron variant (B.1.1.529) of SARS-CoV-2: mutational impacts, concerns, and the possible solutions. *Ann Med Surg*. 2022;78:103737. doi:10.1016/j.amsu.2022.103737
11. Song P, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. *Clin Chim Acta*. 2020;509:280–287. doi:10.1016/j.cca.2020.06.017
12. Nagant C, Ponthieux F, Smet J, et al. A score combining early detection of cytokines accurately predicts COVID-19 severity and intensive care unit transfer. *Int J Infect Dis*. 2020;101:342–345. doi:10.1016/j.ijid.2020.10.003
13. Lippi G, Plebani M. Cytokine “storm”, cytokine “breeze”, or both in COVID-19? *Clin Chem Lab Med*. 2021;59(4):637–639. doi:10.1515/cclm-2020-1761
14. Boehm E, Kronig I, Neher RA, et al. Novel SARS-CoV-2 variants: the pandemics within the pandemic. *Clin Microbiol Infect*. 2021;27:1109–1117.
15. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the ‘Cytokine Storm’ in COVID-19. *J Infect*. 2020;80:607–613. doi:10.1016/j.jinf.2020.03.037
16. Cao B, Wang Y, Wen D, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med*. 2020;382(19):1787–1799. doi:10.1056/NEJMoa2001282
17. Stone JH, Frigault MJ, Serling-Boyd NJ, et al. Efficacy of tocilizumab in patients hospitalized with Covid-19. *N Engl J Med*. 2020;383(24):2333–2344. doi:10.1056/NEJMoa2028836
18. Bakhiet M, Taurin S. SARS-CoV-2: targeted managements and vaccine development. *Cytokine Growth Factor Rev*. 2021;58:16–29. doi:10.1016/j.cytogfr.2020.11.001
19. Klassen SA, Senefeld JW, Senese KA, et al. Convalescent plasma therapy for COVID-19: a graphical mosaic of the worldwide evidence. *Front Med*. 2021;8. doi:10.3389/fmed.2021.684151
20. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. 2021;19(7):409–424. doi:10.1038/s41579-021-00573-0
21. Wouters OJ, Shadlen KC, Salcher-Konrad M, et al. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. *Lancet Lond Engl*. 2021;397(10278):1023–1034. doi:10.1016/S0140-6736(21)00306-8
22. Tharmarajah E, Buazon A, Patel V, et al. IL-6 inhibition in the treatment of COVID-19: a meta-analysis and meta-regression. *J Infect*. 2021;82(5):178–185. doi:10.1016/j.jinf.2021.03.008
23. Cron RQ. COVID-19 cytokine storm: targeting the appropriate cytokine. *Lancet Rheumatol*. 2021;3(4):e236–e237. doi:10.1016/S2665-9913(21)00011-4
24. Reeves HM, Winters JL. The mechanisms of action of plasma exchange. *Br J Haematol*. 2014;164(3):342–351. doi:10.1111/bjh.12629
25. Tabibi S, Tabibi T, Conic RRZ, Banisaeed N, Streiff MB. Therapeutic plasma exchange: a potential management strategy for critically ill COVID-19 patients. *J Intensive Care Med*. 2020;35(9):827–835. doi:10.1177/0885066620940259
26. Biçim S, Berber İ, Ali Erkurt M, et al. The prophylactic treatment of COVID-19 in a renal failure and Hodgkin lymphoma patient with convalescent plasma. *IJR2H*. 2022; 5 (2) : 1–5.
27. Beraud M, Goodhue Meyer E, Lozano M, Bah A, Vassallo R, Brown BL. Lessons learned from the use of convalescent plasma for the treatment of COVID-19 and specific considerations for immunocompromised patients. *Transfus Apher Sci*. 2022;61(3):103355. doi:10.1016/j.transci.2022.103355
28. Klassen SA, Senefeld JW, Johnson PW, et al. The effect of convalescent plasma therapy on mortality among patients with COVID-19: systematic review and meta-analysis. *Mayo Clin Proc*. 2021;96(5):1262–1275. doi:10.1016/j.mayocp.2021.02.008
29. Nabil A, Uto K, Elshemy MM, et al. Current coronavirus (SARS-CoV-2) epidemiological, diagnostic and therapeutic approaches: an updated review until June 2020. *EXCLI J*. 2020;19:992–1016. doi:10.17179/excli2020-2554
30. Keller MA, Stiehm ER. Passive immunity in prevention and treatment of infectious diseases. *Clin Microbiol Rev*. 2000;13(4):602–614. doi:10.1128/CMR.13.4.602
31. Casadevall A, Dadachova E, Pirofski L. Passive antibody therapy for infectious diseases. *Nat Rev Microbiol*. 2004;2(9):695–703. doi:10.1038/nrmicro974

32. Rejeki MS, Sarnadi N, Wihastuti R, et al. Convalescent plasma therapy in patients with moderate-to-severe COVID-19: a study from Indonesia for clinical research in low- and middle-income countries. *eClinicalMedicine*. 2021;36:100931. doi:10.1016/j.eclinm.2021.100931
33. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of argentine haemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet Lond Engl*. 1979;314(8154):1216–1217. doi:10.1016/S0140-6736(79)92335-3
34. Beigel JH, Tebas P, Elie-Turenne M-C, et al. Immune plasma for the treatment of severe influenza: an open-label, multicentre, Phase 2 randomised study. *Lancet Respir Med*. 2017;5(6):500–511. doi:10.1016/S2213-2600(17)30174-1
35. Arnold Egloff SA, Junglen A, Restivo JSA, et al. Convalescent plasma associates with reduced mortality and improved clinical trajectory in patients hospitalized with COVID-19. *J Clin Invest*. 2021;131(20):e151788. doi:10.1172/JCI151788
36. Joyner MJ, Bruno KA, Klassen SA, et al. Safety Update: COVID-19 convalescent plasma in 20,000 hospitalized patients. *Mayo Clin Proc*. 2020;95(9):1888–1897. doi:10.1016/j.mayocp.2020.06.028
37. Cohn CS, Estcourt L, Grossman BJ, et al. COVID –19 convalescent plasma: interim recommendations from the AABB. *Transfusion*. 2021;61(4):1313–1323. doi:10.1111/trf.16328
38. Bloch EM, Tobian AAR, Shoham S, et al. How do I implement an outpatient program for the administration of convalescent plasma for COVID –19? *Transfusion*. 2022;62(5):933–941. doi:10.1111/trf.16871
39. Marano G, Vaglio S, Pupella S, et al. Convalescent plasma: new evidence for an old therapeutic tool? *Blood Transfus Trasfus Sanguie*. 2016;14:152–157.
40. McGuire LW, Redden WR. The use of convalescent human serum in influenza pneumonia—a preliminary report. *Am J Public Health*. 1918;8(10):741–744. doi:10.2105/AJPH.8.10.741
41. Cheng Y, Wong R, Soo YOY, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis*. 2005;24(1):44–46. doi:10.1007/s10096-004-1271-9
42. Hung IF, To KK, Lee C-K, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. *Clin Infect Dis*. 2011;52(4):447–456. doi:10.1093/cid/ciq106
43. van Griensven J, Edwards T, de Lamballerie X, et al. Evaluation of convalescent plasma for Ebola virus disease in Guinea. *N Engl J Med*. 2016;374(1):33–42. doi:10.1056/NEJMoa1511812
44. Imai M, Iwatsuki-Horimoto K, Hatta M, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc Natl Acad Sci U S A*. 2020;117(28):16587–16595. doi:10.1073/pnas.2009799117
45. Sun J, Zhuang Z, Zheng J, et al. Generation of a broadly useful model for COVID-19 pathogenesis, vaccination, and treatment. *Cell*. 2020;182(3):734–743.e5. doi:10.1016/j.cell.2020.06.010
46. Chan JF-W, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin Infect Dis*;2020. ciaa325. doi:10.1093/cid/ciaa325
47. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature*. 2021;590(7847):630–634. doi:10.1038/s41586-020-03041-6
48. Cross RW, Prasad AN, Borisevich V, et al. Use of convalescent serum reduces severity of COVID-19 in nonhuman primates. *Cell Rep*. 2021;34(10):108837. doi:10.1016/j.celrep.2021.108837
49. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci*. 2020;117(17):9490–9496. doi:10.1073/pnas.2004168117
50. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA*. 2020;323(16):1582–1589. doi:10.1001/jama.2020.4783
51. Zhang B, Liu S, Tan T, et al. Treatment with convalescent plasma for critically ill patients with severe acute respiratory syndrome coronavirus 2 infection. *Chest*. 2020;158(1):e9–e13. doi:10.1016/j.chest.2020.03.039
52. Cognasse F, Hamzeh-Cognasse H, Rosa M, et al. Inflammatory markers and auto-abs to type I IFNs in COVID-19 convalescent plasma; 2022;87:104414.
53. Casadevall A, Pirofski L-A. The convalescent sera option for containing COVID-19. *J Clin Invest*. 2020;130(4):1545–1548. doi:10.1172/JCI138003
54. Seghatchian J, Lanza F. Convalescent plasma, an apheresis research project targeting and motivating the fully recovered COVID 19 patients: a rousing message of clinical benefit to both donors and recipients alike. *Transfus Apher Sci*. 2020;59(3):102794. doi:10.1016/j.transci.2020.102794
55. Pool JG, Hershgold EJ, Pappenhagen AR. High-potency antihaemophilic factor concentrate prepared from cryoglobulin precipitate. *Nature*. 1964;203(4942):312. doi:10.1038/203312a0
56. Franchini M. Convalescent plasma therapy for managing infectious diseases: a narrative review. *Ann Blood*. 2021;6:17. doi:10.21037/aob-2020-cp-03
57. Psaltopoulou T, Sergentanis TN, Pappa V, et al. The emerging role of convalescent plasma in the treatment of COVID-19. *HemaSphere*. 2020;4(3):e409. doi:10.1097/HS9.0000000000000409
58. World Health Organization. *Use of Convalescent Whole Blood or Plasma Collected from Patients Recovered from Ebola Virus Disease for Transfusion, as an Empirical Treatment During Outbreaks: Interim Guidance for National Health Authorities and Blood Transfusion Services*. World Health Organization; 2014.
59. Luke TC, Kilbane EM, Jackson JL, Hoffman SL. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med*. 2006;145(8):599–609. doi:10.7326/0003-4819-145-8-200610170-00139
60. Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis*. 2015;211(1):80–90. doi:10.1093/infdis/jiu396
61. Arabi Y, Balkhy H, Hajeer AH, et al. Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. *SpringerPlus*. 2015;4(1):709. doi:10.1186/s40064-015-1490-9
62. Park WH, Freeman RG Jr. The prophylactic use of measles convalescent serum. *J Am Med Assoc*. 1926;87(8):556–558. doi:10.1001/jama.1926.02680080022009
63. Park WH. Therapeutic use of antipoliomyelitis serum in preparalytic cases of poliomyelitis. *J Am Med Assoc*. 1932;99(13):1050–1053. doi:10.1001/jama.1932.02740650008003

64. Rambar AC. Mumps; use of convalescent serum in the treatment and prophylaxis of orchitis. *Am J Dis Child*. 1946;71(1):1–13. doi:10.1001/archpedi.1946.0202040008001
65. Contreras-Barbeta E, Millan A, Rello J. Convalescent plasma for SARS-CoV-2 infection: win or learn. *Eur Respir J*. 2022;59(2):2102076. doi:10.1183/13993003.02076-2021
66. Brosig AM, Ossner T, Pamler I, et al. Multistep screening and selection of COVID-19 convalescent plasma donors at the early stage of the SARS-CoV-2 pandemic: a retrospective analysis. *Health Sci Rep*. 2022;5(5):e815. doi:10.1002/hsr2.815
67. Chen B, Xia R. Early experience with convalescent plasma as immunotherapy for COVID-19 in China: knowns and unknowns. *Vox Sang*. 2020;115(6):507–514. doi:10.1111/vox.12968
68. Soo YOY, Cheng Y, Wong R, et al. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. *Clin Microbiol Infect*. 2004;10(7):676–678. doi:10.1111/j.1469-0691.2004.00956.x
69. Duan K, Liu B, Li C, et al. The feasibility of convalescent plasma therapy in severe COVID-19 patients: a pilot study. *MedRxiv*. 2020;2020.2020. doi:10.1101/2020.09.15.20191591
70. Cristelli MP, Langhi Junior DM, Viana LA, et al. Efficacy of convalescent plasma to treat mild to moderate COVID-19 in kidney transplant patients: a propensity score matching analysis. *Transplantation*. 2022;106:e92–e94. doi:10.1097/TP.0000000000003962
71. Budhai A, Wu AA, Hall L, et al. How did we rapidly implement a convalescent plasma program? *Transfusion*. 2020;60:1348–1355. doi:10.1111/trf.15910
72. FDA. Investigational COVID-19 convalescent plasma. U.S. Food and drug administration; 2022. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/investigational-covid-19-convalescent-plasma>. Accessed October 18, 2022.
73. Bacal CJO, Maina JW, Nandurkar HH, et al. Blood apheresis technologies – a critical review on challenges towards efficient blood separation and treatment. *Mater Adv*. 2021;2:7210–7236. doi:10.1039/D1MA00859E
74. Tiberghien P, de Lamballerie X, Morel P, Gallian P, Lacombe K, Yazdanpanah Y. Collecting and evaluating convalescent plasma for COVID-19 treatment: why and how? *Vox Sang*. 2020;115:488–494. doi:10.1111/vox.12926
75. Jones JM, Faruqi AJ, Sullivan JK, Calabrese C, Calabrese LH. COVID-19 outcomes in patients undergoing b cell depletion therapy and those with humoral immunodeficiency states: a scoping review. *Pathog Immun*. 2021;6:76–103. doi:10.20411/pai.v6i1.435
76. Apostolidis SA, Kakara M, Painter MM, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med*. 2021;27(11):1990–2001. doi:10.1038/s41591-021-01507-2
77. Honjo K, Russell RM, Li R, et al. Convalescent plasma-mediated resolution of COVID-19 in a patient with humoral immunodeficiency. *Cell Rep Med*. 2021;2(1):100164. doi:10.1016/j.xcrm.2020.100164
78. Dunbar NM, Yazer MH. Biomedical Excellence for Safer Transfusion (BEST) collaborative and the STAT study investigators. Safety of the use of group A plasma in trauma: the STAT study. *Transfusion*. 2017;57(8):1879–1884. doi:10.1111/trf.14139
79. Bilir Y, Bilir A, Ciyiltepe F, Bombaci E, Saraçoğlu A, Saraçoğlu KT. Comparison of the effectiveness of early and late convalescent plasma treatment given in patients diagnosed with coronavirus disease in intensive care unit. *Kocaeli Med J*. 2022;11(1):43–51. doi:10.5505/ktd.2022.39260
80. Wang P, Liu L, Nair MS, et al. SARS-CoV-2 neutralizing antibody responses are more robust in patients with severe disease. *Emerg Microbes Infect*. 2020;9:2091–2093. doi:10.1080/22221751.2020.1823890
81. Del Fante C, Franchini M, Baldanti F, et al. A retrospective study assessing the characteristics of COVID-19 convalescent plasma donors and donations. *Transfusion*. 2021;61:830–838.
82. Wu -B-B, Gu D-Z, Yu J-N, Yang J, Shen W-Q. Association between ABO blood groups and COVID-19 infection, severity and demise: a systematic review and meta-analysis. *Infect Genet Evol*. 2020;84:104485. doi:10.1016/j.meegid.2020.104485
83. Franchini M, Glingani C, Del Fante C, et al. The protective effect of O blood type against SARS-CoV-2 infection. *Vox Sang*. 2021;116:249–250.
84. Liumbruno GM, Marano G, Grazzini G, Capuzzo E, Franchini M. Solvent/detergent-treated plasma: a tale of 30 years of experience. *Expert Rev Hematol*. 2015;8(3):9490–9496. doi:10.1586/17474086.2015.1016906
85. Focosi D, Franchini M, Pirofski L, et al. COVID-19 convalescent plasma and clinical trials: understanding conflicting outcomes. *Clin Microbiol Rev*. 2022;35(3):e00200–e00221. doi:10.1128/cmr.00200-21
86. Cacilhas P, Caberlon E, Angoleri L, Fassina K, Ribeiro RN, Pinto LC. Convalescent plasma therapy in COVID-19 patients: a non-randomized case-control study with concurrent control. *Braz J Med Biol Res*. 2022;55:1.
87. Libster R, Pérez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe Covid-19 in older adults. *N Engl J Med*. 2021;384(7):610–618. doi:10.1056/NEJMoa2033700
88. Simonovich VA, Burgos Pratz LD, Scibona P, et al. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. *N Engl J Med*. 2021;384(7):619–629. doi:10.1056/NEJMoa2031304
89. Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P. Convalescent plasma in the management of moderate covid-19 in adults in India: open label Phase II multicentre randomised controlled trial (PLACID trial). *BMJ*. 2020;371:m3939. doi:10.1136/bmj.m3939
90. Baker JV, Lane HC. The fast and the furious: chasing a clinical niche for COVID-19 convalescent plasma. *Ann Intern Med*. 2022;175(9):1332–1334. doi:10.7326/M22-2329
91. Hammond J, Leister-Tebbe H, Gardner A, et al. Oral nirmatrelvir for high-risk, nonhospitalized adults with Covid-19. *N Engl J Med*. 2022;386(15):115–408. doi:10.1056/NEJMoa2118542
92. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe*. 2016;19(2):695–703. doi:10.1016/j.chom.2016.01.007
93. Sullivan DJ, Gebo KA, Shoham S, et al. Early outpatient treatment for Covid-19 with convalescent plasma. *N Engl J Med*. 2022;386(18):44–46. doi:10.1056/NEJMoa2119657
94. Millat-Martinez P, Gharbharan A, Alemany A, et al. Prospective individual patient data meta-analysis of two randomized trials on convalescent plasma for COVID-19 outpatients. *Nat Commun*. 2022;13(1):2583. doi:10.1038/s41467-022-29911-3
95. Focosi D, Franchini M. Potential use of convalescent plasma for SARS-CoV-2 prophylaxis and treatment in immunocompromised and vulnerable populations. *Expert Rev Vaccines*. 2022;21(7):877–884. doi:10.1080/14760584.2021.1932475
96. Hueso T, Godron A-S, Lanoy E, et al. Convalescent plasma improves overall survival in patients with B-cell lymphoid malignancy and COVID-19: a longitudinal cohort and propensity score analysis. *Leukemia*. 2022;36(4):1025–1034. doi:10.1038/s41375-022-01511-6

97. Focosi D, Franchini M, Joyner MJ, Casadevall A, Sullivan DJ. Analysis of anti-omicron neutralizing antibody titers in different convalescent plasma sources. *medRxiv*. 2022;2021. doi:10.1101/2021.12.24.21268317
98. Rijnders BJA, Huygens S, Mitjà O. Evidence-based dosing of convalescent plasma for COVID-19 in future trials. *Clin Microbiol Infect*. 2022;28:667–671. doi:10.1016/j.cmi.2022.01.026
99. Belcarì G, Conti A, Mazzoni A, Lanza M, Mazzetti P, Focosi D. Clinical and virological response to convalescent plasma in a chronic lymphocytic leukemia patient with COVID-19 Pneumonia. *Life*. 2022;12:1098. doi:10.3390/life12071098
100. Hegerova L, Gooley TA, Sweerus KA, et al. Use of convalescent plasma in hospitalized patients with COVID-19: case series. *Blood*. 2020;136:759–762. doi:10.1182/blood.202006964
101. Liu STH, Lin H-M, Baine I, et al. Convalescent plasma treatment of severe COVID-19: a propensity score-matched control study. *Nat Med*. 2020;26:1708–1713. doi:10.1038/s41591-020-1088-9
102. Salazar E, Christensen PA, Graviss EA, et al. Treatment of coronavirus disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality. *Am J Pathol*. 2020;190(11):2290–2303. doi:10.1016/j.ajpath.2020.08.001
103. Abolghasemi H, Eshghi P, Cheraghali AM, et al. Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: results of a multicenter clinical study. *Transfus Apher Sci*. 2020;59(5):102875. doi:10.1016/j.transci.2020.102875
104. Belov A, Huang Y, Villa CH, et al. Early administration of COVID-19 convalescent plasma with high titer antibody content by live viral neutralization assay is associated with modest clinical efficacy. *Am J Hematol*. 2022;97:770–779. doi:10.1002/ajh.26531
105. Park H, Tarpey T, Liu M, et al. Development and validation of a treatment benefit index to identify hospitalized patients with COVID-19 who may benefit from convalescent plasma. *JAMA Netw Open*. 2022;5(1):e2147375. doi:10.1001/jamanetworkopen.2021.47375
106. Pardiwalla B, Yadav A, Bhatia A, et al. Convalescent plasma to limit coronavirus associated complications: a proof of concept phase-2 clinical study at a designated COVID-19 hospital in Mumbai city. *J Curr Med Res Opin*. 2021;4(05):940–949. doi:10.15520/jcmro.v4i05.419
107. Arabi YM, Hajeer AH, Luke T, et al. Feasibility of using convalescent plasma immunotherapy for MERS-CoV infection, Saudi Arabia. *Emerg Infect Dis*. 2016;22(9):1554–1561. doi:10.3201/eid2209.151164
108. Ko J-H, Seok H, Cho SY, et al. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. *Antivir Ther*. 2018;23(7):617–622. doi:10.3851/IMP3243
109. Zhang J-S, Chen J-T, Liu Y-X, et al. A serological survey on neutralizing antibody titer of SARS convalescent sera. *J Med Virol*. 2005;77(2):147–150. doi:10.1002/jmv.20431
110. Yeh K-M, Chiueh T-S, Siu LK, et al. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. *J Antimicrob Chemother*. 2005;56(5):919–922. doi:10.1093/jac/dki346
111. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest*. 2020;130(6):2757–2765. doi:10.1172/JCI138745
112. Franchini M, Liumbruno GM, Piacentini G, Glingani C, Zaffanello M. The three pillars of COVID-19 convalescent plasma therapy. *Life*. 2021;11(4):354. doi:10.3390/life11040354
113. Bloch EM, Goel R, Wendel S, et al. Guidance for the procurement of COVID-19 convalescent plasma: differences between high- and low-middle-income countries. *Vox Sang*. 2021;116(1):18–35. doi:10.1111/vox.12970
114. Pouladzadeh M, Safdarian M, Eshghi P, et al. A randomized clinical trial evaluating the immunomodulatory effect of convalescent plasma on COVID-19-related cytokine storm. *Intern Emerg Med*. 2021;16(8):2181–2191. doi:10.1007/s11739-021-02734-8
115. Rogers R, Shehadeh F, Mylona EK, et al. Convalescent plasma for patients with severe Coronavirus Disease 2019 (COVID-19): a matched cohort study. *Clin Infect Dis*. 2021;73(1):e208–e214. doi:10.1093/cid/ciaa1548
116. Shenoy AG, Hettinger AZ, Fernandez SJ, Blumenthal J, Baez V. Early mortality benefit with COVID-19 convalescent plasma: a matched control study. *Br J Haematol*. 2021;192(4):706–713. doi:10.1111/bjh.17272
117. Moniuszko-Malinowska A, Czupryna P, Zarębska-Michaluk D, et al. Convalescent plasma transfusion for the treatment of COVID-19—experience from Poland: a multicenter study. *J Clin Med*. 2020;10(1):E28. doi:10.3390/jcm10010028
118. Joyner MJ, Carter RE, Senefeld JW, et al. Convalescent plasma antibody levels and the risk of death from Covid-19. *N Engl J Med*. 2021;384:1015–1027. doi:10.1056/NEJMoa2031893
119. Korley FK, Durkalski-Mauldin V, Yeatts SD, et al. Early convalescent plasma for high-risk outpatients with Covid-19. *N Engl J Med*. 2021;385:1951–1960. doi:10.1056/NEJMoa2103784
120. Bégin P, Callum J, Jamula E, et al. Convalescent plasma for hospitalized patients with COVID-19: an open-label, randomized controlled trial. *Nat Med*. 2021;27:2012–2024. doi:10.1038/s41591-021-01488-2
121. Delgado-Fernández M, García-Gemar GM, Fuentes-López A, et al. Treatment of COVID-19 with convalescent plasma in patients with humoral immunodeficiency - three consecutive cases and review of the literature. *Enferm Infecc Microbiol Clin*. 2021;40:507–516.
122. Gupta A, Kute VB, Patel HV, et al. Feasibility of convalescent plasma therapy in kidney transplant recipients with severe COVID-19: a single-center prospective cohort study. *Exp Clin Transplant*. 2021;19:304–309.
123. Rodionov RN, Biener A, Spieth P, et al. Potential benefit of convalescent plasma transfusions in immunocompromised patients with COVID-19. *Lancet Microbe*. 2021;2(4):e138. doi:10.1016/S2666-5247(21)00030-6
124. Bennett-Guerrero E, Romeiser JL, Talbot LR, et al. Severe acute respiratory syndrome coronavirus 2 convalescent plasma versus standard plasma in coronavirus disease 2019 infected hospitalized patients in New York: a double-blind randomized trial. *Crit Care Med*. 2021;49(7):1015–1025. doi:10.1097/CCM.0000000000005066
125. Bainbridge ED, Hsue PY, Esensten JH, et al. Characteristics of high-titer convalescent plasma and antibody dynamics after administration in patients with severe coronavirus disease 2019. *Open Forum Infect Dis*. 2021;8(8):ofab385. doi:10.1093/ofid/ofab385
126. Sekine L, Arns B, Fabro BR, et al. Convalescent plasma for COVID-19 in hospitalised patients: an open-label, randomised clinical trial. *Eur Respir J*. 2022;59(2):2101471. doi:10.1183/13993003.01471-2021
127. Khoury E, Nevitt S, Madsen WR, Turtle L, Davies G, Palmieri C. Differences in outcomes and factors associated with mortality among patients with SARS-CoV-2 infection and cancer compared with those without cancer: a systematic review and meta-analysis. *JAMA Netw Open*. 2022;5(5):e2210880. doi:10.1001/jamanetworkopen.2022.10880
128. Ricke DO. Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.640093

129. Wen J, Cheng Y, Ling R, et al. Antibody-dependent enhancement of coronavirus. *Int J Infect Dis.* 2020;100:483–489.
130. Körper S, Weiss M, Zickler D, et al. Results of the CAPSID randomized trial for high-dose convalescent plasma in patients with severe COVID-19. *J Clin Invest.* 2021;131(20):e152264. doi:10.1172/JCI152264
131. Marshall JC, Murthy S, Diaz J, et al. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis.* 2020;20(8):e192–e197. doi:10.1016/S1473-3099(20)30483-7
132. Fung M, Nambiar A, Pandey S, et al. Treatment of immunocompromised COVID-19 patients with convalescent plasma. *Transpl Infect Dis.* 2021;23:e13477.
133. Bhurayanontachai R, Siripaitoon P, Kositpantawong N, Chusri S. The success of non-ABO-identical convalescent plasma transfusion in coronavirus disease 2019 (COVID-19) related acute respiratory distress syndrome (CARDS): a case-report: convalescent plasma for COVID-19. *Clin Crit Care.* 2022;30:e0002–2022. doi:10.54205/cc.v30.254752
134. Roback JD, Guarner J. Convalescent plasma to treat COVID-19: possibilities and challenges. *JAMA.* 2020;323(16):1561–1562. doi:10.1001/jama.2020.4940
135. Sullivan HC, Roback JD. Convalescent plasma: therapeutic hope or hopeless strategy in the SARS-CoV-2 pandemic. *Transfus Med Rev.* 2020;34(3):145–150. doi:10.1016/j.tmr.2020.04.001
136. Amri N, Tessier N, Bégin R, et al. Blood endothelial-cell extracellular vesicles as potential biomarkers for the selection of plasma in COVID-19 convalescent plasma therapy. *Cells.* 2022;11(19):3122. doi:10.3390/cells11193122
137. Bajpai M, Maheshwari A, Kumar S, et al. Comparison of safety and efficacy of convalescent plasma with fresh frozen plasma in severe covid-19 patients. *An Acad Bras Ciênc.* 2022;94. doi:10.1590/0001-376520220210202
138. Bansal N, Raturi M, Bansal Y, Singh P. A novel scoring system for selecting the target patients of COVID-19 convalescent plasma therapy: a hypothesis. *Transfus Clin Biol.* 2022;29:89–91. doi:10.1016/j.tracbi.2021.06.004
139. Bruiners N, Guerrini V, Ukey R, et al. Biologic correlates of beneficial convalescent plasma therapy in a COVID-19 patient reveal disease resolution mechanisms. *medRxiv.* 2022;2022. doi:10.1101/2022.02.03.22269612
140. De Silvestro G, Marson P, La Raja M, et al. Outcome of SARS CoV-2 in patients treated with convalescent plasma: one-year of data from the Veneto region (Italy) Registry. *Eur J Intern Med.* 2022;97:42–49. doi:10.1016/j.ejim.2021.12.023
141. Gullo G, Cucinella G, Tumminello M, et al. Convalescent plasma use in pregnant patients with COVID19 related ARDS: a case report and literature review. *Ital J Gynaecol Obstet.* 2022;34:228. doi:10.36129/jog.2022.32
142. Duan L, Xie Y, Wang Q, et al. Research on antibody changes and nucleic acid clearance in COVID-19 patients treated with convalescent plasma. *Am J Transl Res.* 2022;14:2655–2667.
143. Emma Diletta S, Virginia P, Francesco P, et al. Convalescent plasma therapy in aHUS patient with SARS-CoV-2 infection. *J Clin Nephrol.* 2022;6:9. doi:10.29328/journal.jcn.1001088
144. Erol S, Tanaçan A, Apaydin H, et al. An evaluation of the results of convalescent plasma therapy applied to pregnant women diagnosed as COVID-19- positive in a pandemic center: a prospective cohort study. *Turk J Med Sci.* 2022;52:554–564. doi:10.55730/1300-0144.5346
145. Esteban I, Panighetti MT, Polack FP. Post-trial follow-up after a randomized clinical trial of COVID-19 convalescent plasma. *Gates Open Res.* 2022;6:110. doi:10.12688/gatesopenres.13725.1
146. Freise NF, Gliga S, Fischer J, et al. Convalescent plasma treatment for SARS-CoV-2 infected high-risk patients - a matched pair analysis to the LEOSS cohort. *Sci Rep.* 2022;12(1):19035. doi:10.1038/s41598-022-23200-1
147. Fazeli A, Sharifi S, Behdad F, et al. Early high-titer convalescent plasma therapy in patients with moderate and severe COVID-19. *Transfus Apher Sci.* 2022;61:103321. doi:10.1016/j.transci.2021.103321
148. Fernández-Lázaro D, Ortega CD, Sánchez-Serrano N, et al. Convalescent plasma therapy, therapeutic formulations of repurposed drugs in 20th century epidemics against COVID-19: a systematic review. *Pharmaceutics.* 2022;14:1020. doi:10.3390/pharmaceutics14051020
149. Fernández-Sánchez V, Ventura-Enríquez Y, Cabello-Gutiérrez C, et al. Convalescent plasma to treat Covid-19: a randomized double blind 2 centers trial. *Review.* 2022. doi:10.21203/rs.3.rs-1277990/v1
150. Fodor E, Müller V, Iványi Z, et al. Early transfusion of convalescent plasma improves the clinical outcome in severe SARS-CoV2 infection. *Infect Dis Ther.* 2022;11:293–304. doi:10.1007/s40121-021-00514-7
151. Franchini M, Glingani C, Donno GD, et al. Convalescent plasma for hospitalized COVID-19 patients: a single-center experience. *Life.* 2022;12:420. doi:10.3390/life12030420
152. Franchini M, Focosi D, Percivalle E, et al. Variant of concern-matched COVID-19 convalescent plasma usage in seronegative hospitalized patients. *Viruses.* 2022;14(7):1443. doi:10.3390/v14071443
153. Gharbharan A, GeurtsvanKessel CH, Jordans CCE, et al. Effects of treatment of Coronavirus Disease 2019 with convalescent plasma in 25 B-cell-depleted patients. *Clin Infect Dis.* 2022;74:1271–1274. doi:10.1093/cid/ciab647
154. Jain R, Mallya MV, Amoncar S, et al. Seroprevalence of SARS-CoV-2 among potential convalescent plasma donors and analysis of their deferral pattern: experience from tertiary care hospital in western India. *Transfus Clin Biol.* 2022;29:60–64. doi:10.1016/j.tracbi.2021.07.004
155. Kutsuna S, Saito S, Takamatsu Y, et al. Safety of convalescent plasma therapy for COVID-19 patients and analysis of viral kinetics: a single-center, open-label, single-arm, interventional study in Japan. *GHM Open.* 2022;2(1):38–43.
156. Kurnianda J, Hardianti MS, Triyono T, et al. Efficacy and safety of convalescent plasma therapy in patients with moderate-to-severe COVID-19: a non-randomized comparative study with historical control in a referral hospital in Indonesia. *J Infect Public Health.* 2022;15:100–108. doi:10.1016/j.jiph.2021.10.028
157. Leon J, Merrill AE, Rogers K, et al. SARS-CoV-2 antibody changes in patients receiving COVID-19 convalescent plasma from normal and vaccinated donors. *Transfus Apher Sci.* 2022;61:103326. doi:10.1016/j.transci.2021.103326
158. Magyar F, Pinczés LI, Páyer E, et al. Early administration of remdesivir plus convalescent plasma therapy is effective to treat COVID-19 pneumonia in B-cell depleted patients with hematological malignancies. *Ann Hematol.* 2022;101(10):2337–2345. doi:10.1007/s00277-022-04924-6
159. Marconato M, Abela IA, Hauser A, et al. Antibodies from convalescent plasma promote SARS-CoV-2 clearance in individuals with and without endogenous antibody response. *J Clin Invest.* 2022;132(12). doi:10.1172/JCI158190
160. Mastroianni A, Greco S, Chidichimo L, et al. Challenges associated with convalescent plasma infusion therapy during pregnancy, a case report. *Womens Health Bull.* 2022;9:118–121.

161. Mesina F, Julian J, Relos J, et al. Use of convalescent plasma therapy with Best Available Treatment (BAT) among hospitalized COVID-19 patients: a multi-center study. *medRxiv*. 2022;2022:2022.
162. Nyström K, Hjorth M, Fust R, et al. Specific T-cell responses for guiding treatment with convalescent plasma in severe COVID-19 and humoral immunodeficiency: a case report. *BMC Infect Dis*. 2022;22(1):362. doi:10.1186/s12879-022-07323-4
163. Novacescu AN, Duma G, Buzzi B, et al. Therapeutic plasma exchange followed by convalescent plasma transfusion in severe and critically ill COVID-19 patients: a single centre non-randomized controlled trial. *Exp Ther Med*. 2022;23:1–8. doi:10.3892/etm.2021.10923
164. Novacescu AN, Duma G, Sandesc D, Sorescu T, Licker M. The successful recovery of a critically ill COVID-19 patient, following the combination of therapeutic plasma exchange and convalescent plasma transfusion: a case report. *Medicina*. 2022;58:1088. doi:10.3390/medicina58081088
165. Noor ES, Semedi BP, Kusuma E. The effect of convalescent plasma administration on increasing blood IGG and IGM levels as an effort to prevent death due to Covid-19 in ICU Panglima Sebaya hospital, Paser Regency, East Kalimantan. *J Adv Health Med Sci*. 2022;8. doi:10.20474/jahms-8.1
166. Ostrovskaya EA, Kostin AI, Bulanov A. Efficiency and safety of convalescent plasma therapy in patients with COVID-19: a systematic review. *Ann Crit Care*. 2022;2:108–120.
167. Ortigoza MB, Yoon H, Goldfeld KS, et al. Efficacy and safety of COVID-19 Convalescent plasma in hospitalized patients: a randomized clinical trial. *JAMA Intern Med*. 2022;182:115–126. doi:10.1001/jamainternmed.2021.6850
168. Pan C, Chen H, Xie J, et al. The efficiency of convalescent plasma therapy in the management of critically ill patients infected with COVID-19: a matched cohort study. *Front Med*. 2022;9:822821. doi:10.3389/fmed.2022.822821
169. Pratama A, Djaharuddin I. Effectiveness and efficacy of convalescent plasma therapy in Covid-19 patients. In: *C62. Expanding Our Insight into Covid-19*. American Thoracic Society; 2022:A4568–A4568.
170. Qin J, Wang G, Han D. Benefits of plasma exchange on mortality in patients with COVID-19: a systematic review and meta-analysis. *Int J Infect Dis*. 2022;122:332–336. doi:10.1016/j.ijid.2022.06.014
171. Soleimani Z, Soleimani A. ADRS due to COVID-19 in midterm pregnancy: successful management with plasma transfusion and corticosteroids. *J Matern Fetal Neonatal Med*. 2022;35:3040–3043. doi:10.1080/14767058.2020.1797669
172. Sanz C, Nomdedeu M, Pereira A, et al. Efficacy of early transfusion of convalescent plasma with high-titer SARS-CoV-2 neutralizing antibodies in hospitalized patients with COVID-19. *Transfusion*. 2022;62:974–981. doi:10.1111/trf.16863
173. Shibeel S. Effectiveness of convalescent plasma therapy in COVID-19 patients with haematological malignancies (preprint); 2022.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Von Willebrand Disease Epidemiology, Burden of Illness and Management: A Systematic Review

Ping Du, Aurore Bergamasco, Yola Moride, Françoise Truong Berthoz, Gülden Özen & Spiros Tzivelekis

To cite this article: Ping Du, Aurore Bergamasco, Yola Moride, Françoise Truong Berthoz, Gülden Özen & Spiros Tzivelekis (2023) Von Willebrand Disease Epidemiology, Burden of Illness and Management: A Systematic Review, Journal of Blood Medicine, , 189-208, DOI: [10.2147/JBM.S389241](https://doi.org/10.2147/JBM.S389241)

To link to this article: <https://doi.org/10.2147/JBM.S389241>



© 2023 The Takeda Pharmaceutical Company Limited.



Published online: 02 Mar 2023.



Submit your article to this journal



Article views: 953



View related articles



View Crossmark data



Citing articles: 12 View citing articles

Von Willebrand Disease Epidemiology, Burden of Illness and Management: A Systematic Review

Ping Du ¹, Aurore Bergamasco ², Yola Moride ^{2,3}, Françoise Truong Berthoz⁴, Gülден Özen¹, Spiros Tzivelekis¹

¹Takeda Development Center Americas, Inc., Cambridge, MA, USA; ²YOLARX Consultants, Paris, France; ³Rutgers, The State University of New Jersey, New Brunswick, NJ, USA; ⁴Takeda Pharmaceuticals International AG, Zürich, Switzerland

Correspondence: Ping Du, Takeda Development Center Americas, Inc., 40 Landsdowne Street, Cambridge, MA, 02139, USA, Tel +1-717-307-0572, Email ping.du@takeda.com

Introduction: Although hereditary von Willebrand disease (VWD) is the most common bleeding disorder, its epidemiology is not well understood. A systematic review (PROSPERO CRD42020197674/CRD42021244374) on the epidemiology/burden of illness of VWD was conducted to better understand patients' unmet needs.

Methods: Observational studies (published January 1, 2010 to April 14, 2021) were identified in MEDLINE and Embase databases, using free-text keywords and thesaurus terms for VWD and outcomes of interest. Pragmatic web-based searches of the gray literature, including conference abstracts, were performed, and reference lists of retained publications were manually searched for additional sources. Case reports and clinical trials (phase 1–3) were excluded. Outcomes of interest were incidence, prevalence, mortality, patient characteristics, burden of illness, and therapeutic management/treatments currently used for VWD.

Results: Of the 3095 identified sources, 168 were included in this systematic review. Reported VWD prevalence (22 sources) ranged from 108.9 to 2200 per 100,000 in population-based studies and from 0.3 to 16.5 per 100,000 in referral-based studies. Reported times between first symptom onset and diagnosis (two sources; mean 669 days; median 3 years) highlighted gaps in timely VWD diagnosis. Bleeding events reported in 72–94% of the patients with VWD (all types; 27 sources) were mostly mucocutaneous including epistaxis, menorrhagia, and oral/gum bleeding. Poorer health-related quality of life (three sources) and greater health care resource utilization (three sources) were reported for patients with VWD than in general populations.

Conclusion: Available data suggest that patients with VWD experience high disease burden in terms of bleeding, poor quality of life, and health care resource utilization.

Keywords: disease burden, epidemiology, health-related quality of life, health care resource utilization, systematic review, von Willebrand disease

Introduction

von Willebrand disease (VWD) is an inherited bleeding disorder characterized by defects in the concentration, structure, or function of von Willebrand factor (VWF), a glycoprotein that acts as a carrier protein for factor VIII (FVIII) and facilitates platelet adhesion at vascular injury sites.^{1–3} There are three main types of VWD that differ according to the degree of disease severity and inheritance pattern.² Types 1 and 3 are characterized by quantitative defects in the VWD protein: type 1 VWD is characterized by partial quantitative VWF deficiency, resulting in a mild to moderate bleeding phenotype, whereas type 3, the most severe form of VWD, results from near-complete absence of VWF.^{2,4} Type 2 VWD represents a group of disease phenotypes resulting from qualitative defects in VWF, affecting formation of multimers (types 2A and 2B), platelet adhesion (type 2M), or FVIII binding (type 2N).^{2,4} Bleeding complications in patients with VWD vary depending on the level of residual VWF activity, disease type, age, and sex.⁵ Patients most often experience mucocutaneous bleeding, including epistaxis, easy bruising, and heavy menstrual bleeding (HMB), as well as bleeding after surgery/trauma.^{4,5}

VWD is considered the most common hereditary bleeding disorder,⁶ but information on the epidemiology of VWD is limited. This systematic review was, therefore, conducted to better understand the disease and related unmet needs for the patients. Data on the epidemiology of VWD worldwide were reviewed, including incidence, prevalence, mortality, patient characteristics, natural history, burden of illness, and therapeutic management/treatments received in the real-world clinical practice setting.

Methods

A systematic review was conducted between January 1, 2010 and April 14, 2021 (date last searched) using methods developed by the Cochrane group and the National Academy of Medicine, formerly the Institute of Medicine of the National Academy.^{7,8} This time period was selected to provide comprehensive search results for the outcomes of interest that were also reflective of recent trends in disease burden, epidemiology and clinical management strategies. The protocol was registered under the International Prospective Register of Systematic Reviews. The original protocol, which covered searches through to July 1, 2020, was registered on August 8, 2020 (PROSPERO CRD42020197674). An updated protocol was registered on May 12, 2021, and covered pragmatic searches from July 1, 2020 to April 14, 2021, and literature searches from July 1, 2020 to March 22, 2021, as well as from January 2010 to March 22, 2021, for publications not covered by the original protocol (ie, scientific communications without abstracts) (PROSPERO CRD42021244374).

Search Strategy and Information Sources

Comprehensive literature searches of MEDLINE and Embase were conducted to identify publications written in English. Search strategies were based on free-text keywords and thesaurus terms (ie, Medical Subject Headings [MeSH] and Emtree terms) combining the following concepts: population and data of interest. Search strategies are detailed in [Supplemental Table 1](#). In addition, pragmatic searches were performed using Google, Google Scholar, the Cochrane Library, and websites of relevant scientific societies involved in VWD, as well as abstracts from related conferences ([Supplemental Table 2](#)). The reference lists of retained publications were manually searched for additional relevant sources (“snowballing”).

Eligibility Criteria

Observational (non-interventional) studies, literature reviews, or meta-analyses involving patients with VWD and reporting on epidemiologic, burden of illness, and management data of interest were included. [Table 1](#) provides a complete list of inclusion and exclusion criteria.

Table 1 Eligibility Criteria for the Selection of Sources Included in This Systematic Review

Inclusion Criteria	Exclusion Criteria
• Studies published in English between January 1, 2010 and March 22, 2021 • Studies conducted in humans • Non-interventional (observational) studies • Studies that included patients with von Willebrand disease, either as the study population or as a subgroup analysis • Studies that reported epidemiologic data of interest (epidemiologic parameters, patient characteristics, natural history of disease, burden of illness, disease management) • Original research articles • Full-text articles, letters to the editors, editorials, conference proceedings, abstracts, reports • Literature reviews (systematic or non-systematic) and meta-analyses (both types to be used for snowballing only) • For studies with multiple publications, only the latest publication was retained	• Case reports • Opinions • Clinical trials (phase 1–3) • Non-clinical and experimental studies • Studies reporting preliminary results (later published in full text)

Selection of Studies

During the first stage, titles and/or abstracts were screened using the predefined eligibility criteria by two independent reviewers, with conflicts resolved by a third assessor, and during the second stage, eligibility was confirmed by in-depth review of full texts. Reasons for exclusion at second stage were documented. For studies with multiple publications, only the most recent reporting on each outcome of interest was retained.

Data Extraction and Data Synthesis

Data from relevant publications were extracted into a standardized data extraction form and the extracted data evaluated for completeness. Data items extracted included general information (source, citation, publication type, geographical coverage); study methods (study period, study design, data source); target population (age); VWD type; study population (diagnostic criteria); number of patients with VWD; and definition of the reference population. The following study outcomes were also extracted: patient characteristics (sociodemographics and medical history), incidence/prevalence estimates, all-cause and VWD-related mortality, time from symptoms onset to diagnosis, disease severity, disease progression, bleeding events at diagnosis and during follow-up, disease management/treatment used in clinical practice, health care resource utilization (HCRU), and health-related quality of life (HRQoL).

For each study, the methodology was assessed qualitatively. Specific attention was paid to the robustness of study methods and the generalizability of findings, and these results were used to inform data synthesis. Risk-of-bias tools were not used.

Results of the systematic review were synthesized qualitatively; there was no pooling of estimates through a meta-analysis. For prevalence, incidence, and mortality, a range of estimates was reported according to country/region. Ranges of estimates were provided, and outliers identified using qualitative appraisal and reason for outlying estimates qualitatively assessed; no statistical tests were conducted. Details regarding characteristics of patients and natural history of disease were reported according to VWD type, if available. The burden of illness was described in terms of HCRU and impact of the disease on patients' HRQoL.

Results

Study Selection

A total of 3095 references were identified through the combined search of electronic bibliographical databases (Medline $n = 735$, Embase $n = 2360$). Following removal of duplicates, titles/abstracts of 2671 unique references ([Supplemental Table 1](#)) were screened independently by two assessors and 2368 references were excluded. Of the 303 full-text articles assessed for eligibility, 136 met the eligibility criteria; a further 32 references were identified through pragmatic searches ([Supplemental Table 2](#)). Thus, 168 sources (ie, unique references) ([Supplemental Table 3](#)) were retained for this systematic review ([Figure 1](#)).

VWD Epidemiology

Prevalence

Estimates of the prevalence of VWD (overall or by specific type) were available from 22 of the 168 sources and varied according to the method of estimation. Referral-based estimates were derived from the number of symptomatic patients attending hemophilia treatment centers or health care facilities based on national registries or surveillance studies. Population-based estimates relied on the identification of patients with VWD in cohort studies based on specific diagnostic criteria.

Multinational estimates for VWD (all types) were reported from two global sources, and a cross-sectional study conducted in Western Europe and Israel. Based on expert opinion, Orphanet reported that globally 0.6–1.3% of the general population was affected by VWD (unspecified data source and time period), and that the prevalence of symptomatic VWD was approximately 10 per 100,000.⁹ The 2019 annual global survey of the World Federation of Hemophilia yielded a referral-based estimate of 1.5 per 100,000 based on the number of patients (with all types of VWD)

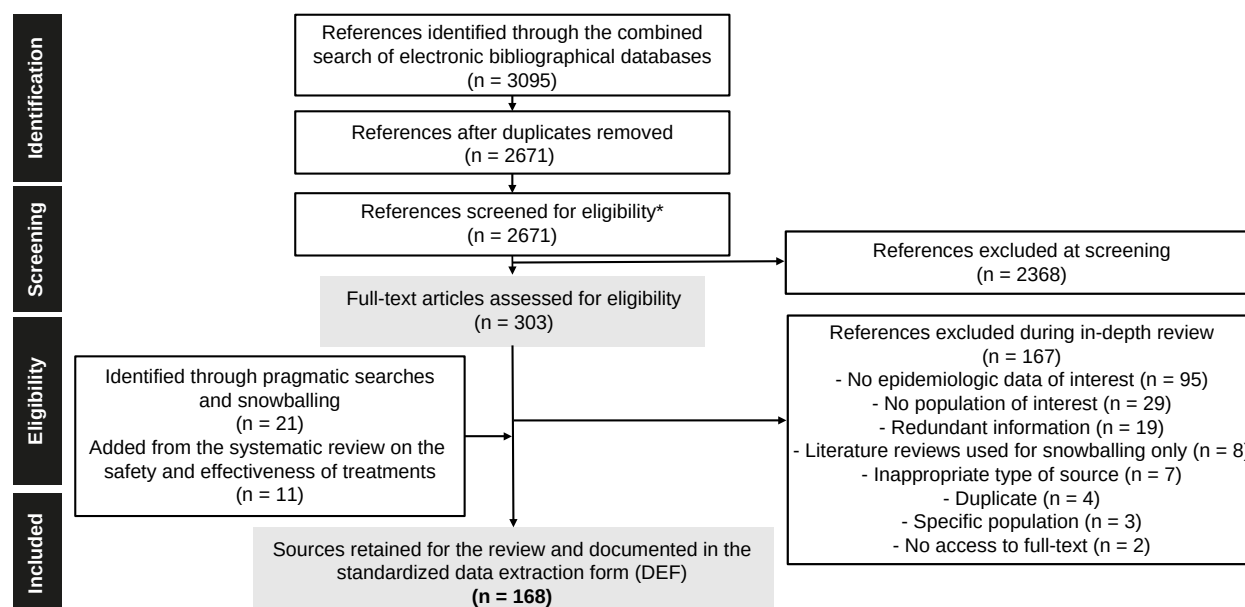


Figure 1 Flow chart for the identification and selection of published sources included for this systematic review. *Independently by two assessors.

registered in hemophilia treatment centers.¹⁰ The European cross-sectional study yielded a population-based estimate of 0.05 per 100,000 for type 3 VWD.¹¹

Country-specific prevalence estimates for VWD were reported from 17 sources: five from Europe, three from Americas, and nine from other regions (Table 2). Referral-based prevalence estimates for overall VWD ranged from 4.4 to 16.5 per 100,000 in Europe,^{12–16} from 0.1 to 8.8 per 100,000 in Western Pacific,^{17–21} and from 1.8 to 2.0 per 100,000 in Eastern Mediterranean^{22,23} (Table 2). Referral-based data were also available for the Czech Republic²⁴ and the USA;²⁵ however, as sizes of the catchment populations were not reported, prevalence could not be estimated.

Estimates by VWD type were available from two registries in Europe (Italy¹² and the UK¹⁵) and one registry in the Western Pacific (Australia^{18,26}). Each of these sources reported higher prevalence estimates for type 1 disease than type 2 or type 3: 2.7–7.2 per 100,000 for type 1 VWD, 0.8–2.5 per 100,000 for type 2, and 0.1–0.3 per 100,000 for type 3. Higher prevalence estimates were also reported for adults than for children in the National Register of Congenital Coagulopathies in Italy.¹²

Population-based estimates, which were mostly conducted in pediatric populations, were higher than referral-based estimates, varying between 800 and 1000 per 100,000 in Europe,^{27,28} 1500 per 100,000 in Eastern Mediterranean,²⁹ 108.9 and 1300 per 100,000 in the Americas,^{30,31} and 2200 per 100,000 in Africa.³²

Incidence

Referral-based incidence estimates of VWD according to the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) report, based on the National Hemophilia Database, were 0.6 (all subtypes), 0.3 (type 1 VWD), 0.2 (type 2 VWD), and 0.01 (type 3 VWD) per 100,000.¹⁵ There were no incidence estimates found for the Americas, Eastern Mediterranean, Western Pacific, or Africa regions. The Orphanet database reported that the incidence rate of type 3 VWD varied across countries from 0.05 to 0.1 per 100,000 person-years in Europe and the USA; sources of estimates and time period were not reported.³³

Patient Demographic Characteristics

Reported demographic characteristics of patients with VWD are presented in Table 3. Patients diagnosed with VWD were predominantly white, with a blood group O and a family history of VWD or other bleeding disorder. The proportion of female patients with VWD (all types) ranged from 45% to 68%.^{10,12,13,15,18,19,22,24,25,28–31,34–48}

Table 2 Reported Country-Based Prevalence Estimates of VWD (17 Sources)

Region	Country	Source (Citation)	Prevalence Estimate (per 100,000)	Prevalence Estimation (Time Period)
Referral-based estimates				
Europe	France	FranceCoag Network ^{13,105}	4.4	Based on 2963 patients with VWD (March 31, 2021) and French population of 67,407,241 (January 21, 2021)
	Italy	National Register of Congenital Coagulopathies ^{12,14,16}	- Overall: 5.4 (2016 and 2018) - Adults (>18 years; 2016): - o 5.8 (all types); 4.3 (type 1); 1.2 (type 2); 0.2 (type 3) - Children (≤18 years; 2016): - o 3.6 (all types); 2.7 (type 1); 0.8 (type 2); 0.1 (type 3) - All ages, type 3 (2015): 0.2	
	UK	National Hemophilia Database (NHD) ¹⁵	16.5 (all types); 7.2 (type 1); 2.5 (type 2); 0.3 (type 3)	Based on 11,120 patients diagnosed with VWD (April 2019 to March 2020) and UK population of 67,195,769 in 2020
Americas	US/Canada	Screening program under supervision of National Institute of Health (NIH) ^{11,106}	0.1 (severe VWD)	Time period: 1977 Case definition for severe VWD: • VWF:Ag or plasma ristocetin cofactor levels (VWF:RCo) <10 U/dL or • Bleeding time >20 min and FVIII coagulant activity <5 U/dL
Western Pacific	Australia	Australian Bleeding Disorders registry ^{18,26}	8.8 (all types); 5.6 (type 1); 2.2 (type 2); 0.2 (type 3)	Based on 2221 patients diagnosed with VWD (fiscal year of 2018–2019) and Australian population of 25,365,745 in 2019
	China	Systematic review and meta-analysis ²¹	0.09 (all types)	Time period: 1985–2010
	Japan	Research Committee for the National Surveillance on Coagulation Disorders ^{19,107}	0.7 (all types)	Based on 944 patients diagnosed with VWD (June 11, 2010) and Japanese population of 128,070,000 in 2010
	South Korea	Korea Hemophilia Foundation ^{17,108}	0.3 (all types)	Based on 152 patients diagnosed with VWD (December 31, 2020) and Korean population of 51,709,098 in 2019
	South Korea	Korean Health Insurance Review and Assessment Service (HIRA) database ²⁰	0.1 (all types)	Based on 517 patients with VWD (identified through ICD-10 code D68.0) between 2010 and 2015 and Korean population of 51,529,338
Eastern Mediterranean	Iran	Cross-sectional study using referral data ²³	1.8 (all types)	Based on 50 patients with VWD identified in 3 referral hospitals (September 2009–March 2011)
	Oman	Cross-sectional study ad hoc data collection (EMRs and questionnaire) ²²	2.0 (all types)	101 patients with VWD identified based on ISTH criteria in 2 referral tertiary care hospitals (June 2019)

(Continued)

Table 2 (Continued).

Region	Country	Source (Citation)	Prevalence Estimate (per 100,000)	Prevalence Estimation (Time Period)
Population-based estimates				
Europe	Italy	Prospective cohort study using ad hoc data collection (questionnaires and laboratory values) ²⁷	800	School-aged children (aged 11–14 years) Case definition: - Family history of bleeding - VWF levels below normal range ≥1 other member, on the hemorrhagic side of the subject's family, with low VWF level
	Turkey	Cross-sectional study using ad hoc data collection (questionnaires) ²⁸	1000 (all type I)	Children aged 14–19 years attending high school (October 2006 and March 2007) Case definition: - Decrease of VWF:RCo and/or VWF:Ag - Presence of meaningful bleeding symptoms - Similar symptoms in ≥1 family member according to ISTH criteria
Americas	Canada	Prospective cohort using ad hoc data collection (questionnaire and laboratory values) ³⁰	108.9 (symptomatic VWD)	Unselected children aged ≤18 years attending primary care pediatric clinic (November 2006–June 2008) Case definition: - Significant bleeding symptoms (BS ≥2) - Abnormal VWF laboratory profile
	US	Cross-sectional using ad hoc data collection (questionnaire, medical history and laboratory values) ³¹	1300 (all types)	Healthy children (aged 2–18 years; N=600) attending routine school examination at 2 military hospitals and 1 urban hospital (March 1989–June 1991) Case definition: ≥1 significant bleeding symptom - A family member with bleeding symptoms - VWF activity <2.5 units percentile of the study population distribution
Eastern Mediterranean	Saudi Arabia	Cross-sectional study using ad hoc data collection (questionnaire and laboratory values) ²⁹	1500 among individuals with suspected bleeding disorders	In students aged 17–22 years (February 2014–January 2016) Case definition: Decrease of VWF:Ag/activity and FVIII
Africa	Nigeria	Cross-sectional study using ad hoc data collection ³²	2200 (all types)	VWD identified in 4 of 182 healthy individuals aged 15–35 years Case definition: VWF:Ag and FVIII coagulant levels

Abbreviations: BS, bleeding score; FVIII, factor VIII; ISTH, International Society on Thrombosis and Haemostasis; VWF, von Willebrand factor; VWF:Ag, VWF antigen.

Table 3 Reported Patient Demographic Characteristics

	Overall VWD	Type 1 VWD	Type 2 VWD	Type 3 VWD
Overall age distribution	- Median age: 33–43 years^{13,16,24} - Adult patients (aged ≥18 years): 81.8–84.7%^{15,18} - Majority of patients 11–44 years age group (66%)²⁵ 20–44 years age group (53.3%)¹⁷	- Median age: 33–42 years^{14,24} - Adult patients (aged ≥18 years): 82.5–87.0%^{15,18} - Majority of patients: 11–44 years age group (68%)²⁵	- Median age: 41–46 years^{16,24} - Adult patients (aged ≥18 years): 79.2–80.7%^{15,18} - Majority of patients: 11–44 years age group (58%)²⁵	- Median age: 40–41 years^{16,24} - Adult patients (aged ≥18 years): 71.8–73.5%^{15,18} - Majority of patients: 11–44 years age group (58%)²⁵
Age at symptom onset	Median age at first bleeding event: 1.5–8.5 years^{38,57,62}	Median age at first bleeding event: 2–7 years^{38,57}	Median age at first bleeding event: 1–8 years^{38,57}	Median age at first bleeding event: 0.8–1 year^{38,57}
Age at diagnosis	- Median age at diagnosis: - General population: 32–33 years^{34,52} - Pediatric population: 2.0–5.5 years^{44,57}	- Median age at diagnosis: - General population: 35 years³⁴ - Pediatric population: 3.0–5 years^{44,57}	- Median age at diagnosis: - Pediatric population: 0.8–7.5 years^{40,44,57}	- Median age at diagnosis: - General population: 28 years³⁴ - Pediatric population: 0.8–3 years^{38,44,57,58}
Sex distribution	- Female predominance: 55.3%¹⁰ - Proportion of female patients:^{10,12,13,15,18,19,22,24,25,28–31,34–48} - General population: 45.2–68.2% - Adult population: 60–75.4% - Pediatric population: 43.2–75.8%	Female predominance: 55.3–83.3%^{15,24,25,34,35,38,42,44,53,54,75}	Proportion of female patients: 46.6–58.8%^{15,24,25,34,35,38,40,42,44,54,75}	Proportion of female patients: 41.7–65.8%^{15,18,24,25,34,35,38,42,44,58}
Ethnicity	White: 50–83.1%^{25,31,40,41}	White: 83.9%²⁵	White: 82.0%²⁵	White: 79.0%²⁵
Blood type	Group O: 39.8–87.5%^{24,29–31,37,39,57,62}	Group O: 47.5–93.6%^{24,53,54,62,75}	Group O: 32.0–62.5%^{24,54,62,75}	Group O: 35.3–45.4%^{24,58}
Family history	- Family history of VWD: 13.2–68.9%^{22,23,38,42,55,62,77} - Family history of bleeding: 15.2–60.0% (bleeding disorders)^{40,43,62} 18.9% (hemophilia A)⁶² 51.2% (bleeding tendencies)⁴⁷	- Family history of VWD: 22.4%⁵⁴ - Family history of bleeding: 50% in patients with severe VWD³⁸	- Family history of VWD: 37.5%⁵⁴ - Family history of bleeding: 78.1% in patients with severe VWD³⁸	- Family history of VWD (siblings): 21.1%⁴⁵ - Family history of bleeding: 33.3% in patients with severe VWD³⁸
Consanguinity	36–48.5% in pediatric population (<18 years)^{43,44}	27.7% in pediatric population (<18 years)⁴⁴	25.0% in pediatric population (<18 years)⁴⁴	67–71.9% in pediatric population (<18 years)^{44,56}

Abbreviation: VWD, von Willebrand disease.

Mortality and Causes of Death

Mortality rates were available from seven sources ([Supplemental Table 4](#)),^{15,36,41,44,46,48–51} none of the publications reported mortality according to VWD type. Based on studies conducted in Sweden and the USA, VWD does not appear to be associated with an increased risk of death compared with populations without VWD. In a cohort study including 2790 patients with VWD in Sweden, all-cause mortality over a 22-year observation period (1987–2009) was estimated at 10.8% (equivalent to 235 per 100,000 person-years), and the risk of death in patients with VWD was not statistically different from that of age- and sex-matched individuals without VWD (hazard ratio 0.90; 95% confidence interval [CI], 0.80–1.05).⁴⁹ All-cause mortality in a single-center cohort study (1985–2010) conducted in the USA was 5.1% in 198 patients with VWD compared with 2.0% in 198 patients without VWD matched for age, sex, and cardiovascular comorbidities ($P=0.1$).⁴⁸ A lower inpatient mortality rate was reported for adult patients with VWD (7556 admissions) than for those without VWD (19,918,970 admissions) in a retrospective US cohort study (2009–2011) using inpatient discharge data (1.3% vs 2.2% [$P<0.0001$]).^{41,46} Another retrospective cohort study (2003–2011) using the same database, and focusing on pregnant women, found similar inpatient mortality rates for women with ($n=15,258$) or without VWD ($n=40,602,254$; 0.03% vs 0.02%, respectively [$P=0.970$]).⁵¹

Other studies have reported mortality rates for 11,120 patients with VWD newly registered at hemophilia centers in the UK (0.6% over period of April 2019 to March 2020),¹⁵ 161 children aged <18 years in Turkey (1.2% over period of 1985–2017),⁴⁴ and 506 pregnant women in the USA (0% over period of 2007–2011).⁵⁰

Data on cause of death were limited to two publications. In a retrospective US single-center cohort study (1985–2010), among 10 deaths in 198 patients with VWD, 7 were due to cancer or cancer-related complications.⁴⁸ In the UK, among 69 deaths in patients with VWD ($n=11,120$) recorded in the UKHCDO (2019–2020), carcinoma (7.2%) and bacterial infection (2.9%) were the most common known cause of death; the majority (79.7%) of deaths had an unknown cause.¹⁵

Burden of Illness

In total, 36 sources were identified that described bleeding events and disease-related complications. The burden of illness was also described in eight sources that reported on HRQoL and nine that reported on the HCRU of patients with VWD.

Timing of VWD Diagnosis

The time from onset of symptoms to VWD diagnosis was poorly documented in the literature, with only two sources being identified. One source was a retrospective US cohort study, which was based on administrative claims data ($N=18,182$) and estimated the mean time from the first billing claim for a bleeding event to a VWD diagnosis as 669 days (range, 2–3284 days).⁵² The second source was a European retrospective cohort study based on medical records for 103 patients with severe VWD from 2010 to 2013 in which the estimated median time between the first bleeding event and VWD diagnosis was 3 years (interquartile range [IQR] 0–18).³⁸

Bleeding Events and Disease-Related Complications

Patients with VWD frequently experience bleeding events. Based on data collected from patient questionnaires or chart reviews, between 72% and 94% of the patients had a history of bleeding events.^{40,44,53} Bleeding events commonly occurred at mucocutaneous sites ([Table 4](#)), with epistaxis, HMB, and oral/gum bleeding being the most frequently reported events in the overall VWD population.

HMB occurred frequently, affecting 61–100% of the women of reproductive age with VWD.^{23,31,34,42,44,54–58} Lower estimates (43% and 53%) were found in studies that used medical records.^{47,59} Postpartum hemorrhage occurred less frequently than HMB, occurring in 5.5–12.7% of the deliveries in women with VWD.^{42,47,50,59–63} Miscarriages were reported in 3–29% of the pregnant women with VWD.^{64–66} Rates of anemia and iron deficiency in women with VWD (including pregnant women) ranged from 13% to 21.4%^{50,52,65} and 4.2 to 22.5%.^{67,68}

Wound-related bleeding was also a common non-spontaneous type of bleeding event that was reported in 38–79% of the patients.^{28,34,37,42,54,55,57,58} Post-surgical bleeding events occurred in 2.4–71.9% of the patients with

Table 4 Reported Disease-Related Complications

	Overall VWD	Type 1 VWD	Type 2 VWD	Type 3 VWD
Epistaxis	55–86% ^{23,28,34,42,56,57,62}	38.8–52.9% ^{34,54}	43.8–56.6% ^{34,54}	36.8–77.3% ^{34,45,58}
Hematoma	13.0–71.7% ^{34,37,62}	10.2–11.9% ^{34,54}	10.5% ³⁴	24–51.9% ^{34,56,58}
Wound-related bleeding	38–79% ^{28,34,37,42,54,55,57,58}	35.0–51.0% ^{34,54}	39.1–43.8% ^{34,54}	24–54.5% ^{34,58}
Oral cavity bleeding	37.5–100% ^{31,55,57}	–	–	48–69.9% ^{56,58}
Gum/gingival bleeding ^a	31.9–62.5% ^{23,34,42,45,47,62}	27.0–49.0% ^{34,54}	35.7–56.3% ^{34,54}	57.6% ³⁴
GI bleeding	3.7–24% ^{b23,34,37,42,47,54,59,62,69,70}	4.0–14.3% ^{34,54}	10.3–33.3% ^{34,37,54}	4.6–29% ^{34,45,56,58}
Post-dental extraction bleeding	6.1–84.6% ^{23,28,34,37,43,47,62}	42.0–53.2% ^{34,54}	40.9–60.0% ^{34,54}	40.9–44% ^{34,58}
Post-surgical bleeding	2.4–71.9% ^{31,34,37,47,59}	23.9–35.9% ^{34,54}	21.4–52.3% ^{34,37,54}	28–45.5% ^{34,56,58}
Heavy menstrual bleeding	61–100% ^{23,31,34,42,44,54–58}	79.9–83.3% ^{34,54}	66.7–75.6% ^{34,54}	61.0–69.2% ^{34,56,58}
Postpartum hemorrhage	5.5–12.7% ^{42,47,50,59–63}	30.0% ⁵⁴	20.0% ⁵⁴	15.0–16.0% ^{56,58}
Hemarthrosis ^c	3.8–38% ^{23,37,39,42,44,47,62,73}	7.6% ⁷¹	16.3% ⁷¹	36.6–51% ^{56,58}
Joint bleeding ^c	6.3–32.3% ^{34,39,71–73}	1.9–22.0% ^{34,39,72,73}	7.9–20.6% ^{34,39,72,73}	9.5–47.8% ^{34,39,72,73}
CNS bleeding ^d	1–2% ^{34,37,44,55,69}	0.6% ³⁴	0.8% ³⁴	1.8–9.1% ^{34,56,58}
Anemia	5–58% ^{50,52,65,109,110}	–	–	–
Iron deficiency	4.2–22.5% in females and 4.5% in males ^{67,68}	–	–	–

Notes: ^a9.1% reported in an additional study involving 33 patients.⁴³ ^bIn studies involving adults or patients of all ages; lower rates reported for pediatric patients.^{43,44}

^cSelf-reported by patients in majority of studies. ^dRate of 8.8% (based on the International Society on Thrombosis and Haemostasis Bleeding Assessment Tool [ISTH-BAT] completed by physician) reported in pediatric study in patients with moderate or severe VWD;⁵⁷ other studies based on medical record, International Classification of Diseases, 9th or 10th Revision, codes, or questionnaire.

Abbreviations: CNS, central nervous system; GI, gastrointestinal; VWD, von Willebrand disease.

VWD,^{31,34,37,47,54,56,58,59} with the highest rate observed in patients aged 65–85 years in a sub-analysis of the cross-sectional von Willebrand in the Netherlands (WiN) study.³⁷ Gastrointestinal (GI) bleeding events were reported in 3.7–24% of the overall VWD population, with studies indicating that these events occur less frequently in the pediatric population.^{23,34,37,42–45,47,54,56,58,59,62,69,70} The reported proportions of patients who experienced joint bleeds^{34,39,71–73} or hemarthrosis^{23,37,39,42,44,47,56,58,62,71,73} were heterogenous across the identified studies, ranging from 6.3% to 32.3% and 3.8% to 38%, respectively. Bleeding events occurring in the central nervous system were rarely reported.^{34,37,44,55–58,69}

Bleeding Score

In total, bleeding scores (BSs) in patients with VWD (all types) were reported in eight studies.^{24,37,53,55,57,58,72,74} In the Dutch WiN study (N = 666), the Tosetto BS varied from 10 to 12 in patients with VWD (aged ≥16 years) (normal Tosetto BS score defined as <3).^{37,53,55} In a subgroup analysis of the WiN study that focused on 113 pediatric patients with VWD (aged 0–15 years), the median BS assessed according to the International Society on Thrombosis and Hemostasis Bleeding Assessment Tool (ISTH-BAT) was 9.0 (IQR 5.0–12.5) (normal range defined as <3 in children).⁵⁷ In a Canadian study that included 100 pediatric patients with VWD (aged <18 years), the median (range) Pediatric Bleeding Questionnaire (PBQ) score was estimated to be 7.0 (0–29) (PBQ score of ≥2 is predictive of VWD diagnosis).⁷⁴

BSs were higher/worse in patients with type 2 or 3 than in those with type 1 VWD. In the Dutch WiN study, the median (range) Tosetto BS for patients with either type 1 or type 2 VWD was 10 (–1 to 31) and 13 (0–33), respectively, compared with 19.5 (2–35) in patients with type 3 VWD.⁵⁵ Similarly, in the pediatric subgroup analysis of the WiN study (aged 0–15 years), median (IQR) ISTH-BAT score was 6.5 (4.0–10.0), 10.5 (6.3–12.8), and 17.0 (11.5–25.5) for patients

with type 1, 2, or 3 VWD, respectively.⁵⁷ In the Canadian study, median (range) PBQ score was estimated to be 9 (2–18) for type 1 VWD, 14 (3–17) for type 2 VWD, and 12 (4–29) for type 3 VWD.⁷⁴

Comorbidities

Cardiovascular-Related Comorbidities

Eight sources reported estimates of cardiovascular comorbidities in patients with VWD.^{37,41,46,48,50,64,69,75} The reported proportions of patients with cardiovascular disease (CVD) in the overall VWD population were 15%^{41,46} and 31%³⁷ based on two sources; reported hypertension rates ranged from 20.0% to 64.8% in patients >16 years of age.^{37,41,46,48,64,75} In a retrospective US cohort study based on hospital discharge data, the proportion of patients with at least one CVD or hypertension was lower in patients with (7556 admissions) versus without (19,918,970 admissions) VWD (15% vs 26% [$P < 0.001$] and 37.4% vs 49.4% [$P < 0.0001$], respectively).^{41,46} In a US single-center case-control study involving 198 patients with VWD, atrial fibrillation was reported in 7.6% and hypertension in 37.4% of the patients.⁴⁸ In the Dutch WiN study involving 536 adult patients (aged ≥ 16 years) with type 1 or 2 VWD (type 3 excluded), the comorbidities of hypertension, hypercholesterolemia, and history of arterial thrombotic events were reported in 21.8%, 9.5%, and 4.3% of the patients, respectively.⁷⁵

Digestive System-Related Comorbidities

In a subgroup analysis of the Dutch WiN study focusing on elderly patients with VWD (aged 65–85 years, $n = 71$), 15.5% of the patients had GI diseases.³⁷ In the retrospective US study based on discharge data, the proportion of patients with liver disease was higher in the population of patients with (7556 admissions) versus without (19,918,970 admissions) VWD (1.0% vs 0.8% [$P = 0.0458$]).⁴¹ Chronic liver disease was also reported in 1.9% of the adult patients with type 1 or 2 VWD ($n = 536$) in the WiN study.⁷⁵

Blood-Borne Diseases

The rate of infections with hepatitis B virus in patients with VWD was 0–1.5% across the identified studies.^{23,24,37} Across six studies, the rate of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections in patients with VWD were reported at 0.1–0.4% and 1.1–3.8%, respectively.^{10,12,16,25,41,71} In the retrospective US study, the proportion of patients with HCV was higher in patients with (7556 admissions) versus without (19,918,970 admissions) VWD (3.8% vs 1.8% [$P < 0.0001$]), whereas the proportion with HIV infection was lower in patients with VWD versus without VWD (0.4% vs 0.8% [$P = 0.0001$]).⁴¹

Metabolic and Endocrine Comorbidities

The rates of metabolic and endocrine comorbidities in patients with VWD were reported in five studies.^{41,46,48,50,59,69,75} Rates of hypothyroidism ranged from 2.4% in the Dutch WiN study in patients with type 1 or 2 VWD ($n = 536$)⁷⁵ to 16.2% in the US single-center case-control study involving all types of VWD ($n = 197$); this latter rate was significantly higher than in patients without VWD (5.6%, $P < 0.0001$).^{48,59} In the overall VWD population, reports of diabetes mellitus in three studies ranged from 6.7% to 16.2%,^{41,48,50} whereas 3.7% of the patients with type 1 or 2 VWD ($n = 536$) had diabetes mellitus in the WiN study.⁷⁵ In the retrospective US study, the proportion of patients with diabetes mellitus and obesity was lower in patients with (7556 admissions) versus without (19,918,970 admissions) VWD (13.5% vs 23.4% [$P < 0.0001$] and 9.2% vs 10.0% [$P = 0.028$], respectively).⁴¹

Other Comorbidities

Across three studies, cancer rates ranged from 4.3% (in patients aged ≥ 16 years with type 1 or 2 VWD) to 23.9% (in patients aged 65–85 years with all VWD types); rates based on self-reporting by patients tended to be lower than those based on medical chart review.^{37,75,76} Across the studies identified through the systematic review, none reported on comorbidities involving renal, skin, allergic, or hypersensitivity disorders in patients with VWD.

Health-Related Quality of Life

Eight studies assessed HRQoL in patients with VWD (Table 5).^{39,40,67,73,77–82} VWD was associated with lower/poorer HRQoL scores than reference or general populations in three of these studies. In children aged 0–5 years ($n = 46$) from the Dutch WiN study, mean (SD) scores for general health perception (70 [15] vs 79 [15]) and parental time (83 [27] vs 93 [11]) from the Infant/Toddler Quality of Life (ITQOL) questionnaire were lower than those reported in the reference population (Dutch preschool children without VWD).⁷⁷ Similarly, in children aged 6–15 years ($n = 87$), physical functioning (96 [13] vs 99 [4]), role functioning–emotional/behavioral (95 [15] vs 98 [7]), general health perceptions (76 [20] vs 83 [13]), and physical summary (54 [8] vs 56 [6]) scores from the Child Health Questionnaire Parent Form 50 (CHQ-PF50) and Child Form 87 (CHQ-CF87) were lower than those for the reference population.⁷⁷ A Swedish retrospective study ($N = 29$) reported significantly lower median scores in the 36-item Short Form Health Survey (SF-36) for bodily pain in women with VWD and HMB than in those women from the general population.⁸² In a Canadian cross-sectional study, adult patients (aged ≥ 18 years) with VWD ($N = 102$) were found to have significantly lower scores ($P < 0.05$) in all SF-36 domains (except role–emotional limitations), compared with age- and sex-normative data from the general population.⁸⁰ The physical (PCS) and mental component summary (MCS) scores were also significantly lower (by 2.4 and 3.9 points, respectively [$P < 0.005$]) than the general population.⁸⁰

Data from the Dutch WiN study also showed that within the patient population with VWD ($n = 804$), HRQoL (based on SF-36 scores) was poorer in patients with joint bleeding than in those without these clinical symptoms/conditions (PCS, 73 vs 77 [$P = 0.005$]; MCS, 47 vs 50 [$P = 0.006$]).³⁹ In another analysis from the WiN study, patients with VWD and joint bleeding ($n = 48$) had significantly higher (worse) hemophilia joint health scores (HJHS; median total score 5 [IQR 1–15] vs 1.5 [IQR 0–5]; $P < 0.01$) and more functional limitations on the Hemophilia Activities List (HAL) (median total score 88 [IQR 69–98] vs 100 [IQR 87–100]; $P < 0.01$) than patients with VWD but without joint bleeding ($n = 48$).^{79,81}

Table 5 Reported HRQoL Outcomes in Patients with VWD

	Overall VWD	Type 1 VWD	Type 2 VWD	Type 3 VWD
SF-36 PCS scores	42.3–80.5 ^{39,78,80}	–	–	–
SF-36 MCS scores	45.5–70.0 ^{39,78,80}	–	–	–
HJHS score	–	6 ⁸¹	2.5 ⁸¹	14 ⁸¹
HAL score	–	85 ⁸¹	92 ⁸¹	77 ⁸¹
ITQOL general health perception score ^a	70 ⁷⁷	73 ⁷⁷	70 ⁷⁷	61 ⁷⁷
ITQOL parental time score ^a	83 ⁷⁷	80 ⁷⁷	88 ⁷⁷	73 ⁷⁷
CHQ-PF50 and CHQ-CF87 physical functioning score ^b	96 ⁷⁷	97 ⁷⁷	95 ⁷⁷	92 ⁷⁷
CHQ-PF50 and CHQ-CF87 role functioning – emotional/behavioral score ^b	95 ⁷⁷	97 ⁷⁷	93 ⁷⁷	93 ⁷⁷
CHQ-PF50 and CHQ-CF87 general health perceptions score ^b	76 ⁷⁷	79 ⁷⁷	78 ⁷⁷	54 ⁷⁷
CHQ-PF50 and CHQ-CF87 physical summary score ^b	54 ⁷⁷	55 ⁷⁷	53 ⁷⁷	47 ⁷⁷
Unrestricted physical activity ^c	–	96.6% ⁴⁰	96.5% ⁴⁰	91.6% ⁴⁰
Mean HUI [®] score	0.70 for females 0.77 for males ⁶⁷	–	–	–

Notes: In pediatric populations: ^a0–5 years, ^b6–15 years, ^c2–12 years.

Abbreviations: CHQ-CF87, Child Health Questionnaire Child Form 87; CHQ-PF50, Child Health Questionnaire Parent Form 50; HAL, Hemophilia Activities List; HJHS, hemophilia joint health score; HRQoL, health-related quality of life; HUI, Health Utilities Index; ITQOL, Infant/Toddler Quality of Life; MCS, mental component summary; PCS, physical component summary; SF-36, 36-item Short Form Health Survey; VWD, von Willebrand disease.

Based on data from the Universal Data Collection, a surveillance system funded by the US Centers for Disease Control and Prevention, 4.3% of the children with VWD (aged 2–12 years, $n = 2712$) used a cane, crutches, walker, or wheelchair for mobility assistance, with this rate higher in patients with type 3 VWD than in those with type 1 or 2 (22.6% vs 3.3% or 3.5%, respectively).⁴⁰

Health Care Resource Utilization

The burden of VWD on outpatient/emergency department visits and hospitalizations was reported in nine studies (Table 6). No publications reporting on admissions to intensive care units were identified. In a Swedish population-based case-control study, a twofold increase in the rate of outpatient hospital visits and hospitalizations was recorded in patients with VWD ($n = 2790$) compared with age- and sex-matched controls ($n = 5$ controls per case).⁸³ In this study, the most common causes of outpatient visits were menorrhagia and epistaxis, and GI bleeding was the most common cause of hospitalizations. A retrospective cohort study based on US administrative claims data ($N = 5058$) also showed that patients with VWD and major bleeding events were significantly ($P < 0.0001$) more likely to be admitted to hospital (incidence rate ratio [IRR] 3.2; 95% CI, 2.8–3.8) and to have emergency department (IRR 2.0; 95% CI, 1.8–2.3) and outpatient visits (IRR 1.3; 95% CI, 1.2–1.3) than patients with VWD who had no major bleeding events.⁶⁹ In another retrospective cohort study based on US administrative claims data in patients undergoing major surgery, IRRs for the patients with VWD ($n = 2972$) were 1.2 (95% CI, 1.1–1.2; $P < 0.0001$) for outpatient visits and 1.4 (95% CI, 1.3–1.6; $P < 0.0001$) for emergency department visits in comparison to the patients without VWD.⁸⁴

VWD Disease Management

Data on VWD treatment with an unspecified indication (ie, on-demand, surgery, or prophylaxis) were available from seven sources.^{15,24,34,62,85,86} The main products and doses used in clinical practice are summarized by indication in Table 7. Patterns of on-demand treatment for VWD were reported in 12 sources. On-demand treatments included desmopressin, replacement therapy with VWF (including VWF/FVIII concentrates), antifibrinolytics, and blood transfusion.^{38,51,63,72,85,87–91} There were 17 sources reporting on the use of situational/conditional prophylaxis (ie, for surgical procedures or childbirth), and nine sources on the use of long-term prophylaxis. Treatments for situational/conditional prophylaxis included desmopressin, plasma-derived VWF and VWF/FVIII-containing concentrates (as monotherapy or in combination), recombinant VWF, and tranexamic acid.^{61,73,85,88,92–94} Plasma-derived VWF/FVIII and VWF concentrates were used for long-term prophylaxis (Table 7).^{81,91,92,95–101} The age, sex, and type of VWD of patients receiving long-term prophylaxis varied across studies: median age was 15.9–60 years;^{92,95,96,98,100} more than half were male;^{95,97,99,100} and 3.1–12.5% had VWD type 1, 17.6–46.9% type 2, and 40.6–74.5% type 3.^{92,95–97,99,100,102} Indications for long-term prophylaxis were reported in eight studies: joint bleeding in 13–43.8%, epistaxis or oral bleeding in 23–34.4%, and GI bleeding in 6.3–63.6% of patients with VWD in general, and HMB in 5–21.9% of the women with VWD.^{92,95–100,102}

Discussion

This systematic review shows that there are limited data available on the epidemiology of VWD, indicating the need for more research to be conducted. Prevalence data that were identified varied depending on method, region/country, VWD type, and patient age.^{9–23,27–32} Incidence data were limited to two sources.^{15,33} The systematic review also highlighted high disease burden, with a high proportion of patients with VWD reporting a history of bleeding events.^{23,28,31,34,37,39,42–45,47,54–63,67,68,70–73} Patients with VWD were also reported to have lower/poorer HRQoL scores versus general populations,^{77,80,82} as well as increased HCRU.^{83,84}

VWD prevalence estimates varied highly according to the study populations and method of estimation. Population-based estimates were higher than referral-based estimates (108.9–2200 per 100,000^{27–32} vs 0.1–16.5 per 100,000^{9,10,12–15,17–23}). This is perhaps not surprising given that the population-based studies mostly involved healthy school children or college students (aged ≤ 35 years) or unselected children (aged ≤ 18 years) attending one or more local hospitals.^{27–32} By contrast, the referral-based studies (with the exception of two cross-sectional studies^{22,23}) identified symptomatic patients of all ages in national registry programs or surveillance databases.^{10,12–15,17–20} Therefore, the population-based studies are more likely to identify a greater

Table 6 Reported Outpatient Visits, Emergency Department Visits, and Hospitalizations for Patients with VWD

	Outpatient Visits	Emergency Department Visits	Hospitalizations
≥1 hemophilia treatment center visit per year	68.6% in the pediatric population⁴⁰	–	–
Most common causes of visits	- Menorrhagia: 22.5 visits/1000 PY - Epistaxis: 20.4 visits/1000 PY⁸³	–	–
Incidence rate ratio of visits	- Patients with VWD vs age- and sex-matched controls: 2.3 (95% CI, 2.0–2.7)⁸³ - Patients with vs without VWD undergoing major surgery: 1.2 (95% CI, 1.1–1.2; $P < 0.0001$)⁸⁴ - VWD patients with vs without major bleeding events: 1.3 (95% CI, 1.2–1.3; $P < 0.0001$)⁶⁹	- Patients with vs without VWD undergoing major surgery: 1.4 (95% CI, 1.3–1.6; $P < 0.0001$)⁸⁴ - VWD patients with vs without major bleeding events: 2.0 (95% CI, 1.8–2.3; $P < 0.0001$)⁶⁹	- VWD patients vs age- and sex-matched controls: 2.2 (95% CI, 2.1–2.4)⁸³ - Patients with vs without VWD undergoing major surgery: 1.5 (95% CI, 1.4–1.6; $P < 0.0001$)⁸⁴ - VWD patients with vs without major bleeding events: 3.2 (95% CI, 2.8–3.8; $P < 0.0001$)⁶⁹
Main reasons for admission	–	- Emergency hospitalizations due to menorrhagia: 2/26 (7.7%)⁵⁷ - Traumatic bleeding: 49.0%⁹⁰ - Post-operative bleeding (10.2%)⁹⁰ - Epistaxis (8.7%)⁹⁰	- GI bleeding: 4.1/1000 PY⁸³ - Menorrhagia: 3.5/1000 PY⁸³ - Epistaxis: 3.4/1000 PY⁸³ - Coronary heart disease: 4.0/100 PY³⁶ - Ischemic stroke: 1.8/100 PY³⁶ - Pulmonary embolism: 0.5/100 PY³⁶ - DVT: 0.5/100 PY³⁶ - Surgery: 69.2%³⁷ - Bleeding: 15.4%³⁷ - First bleeding event: 4.9%³⁸
Mean (standard error) length of stay	–	–	4.6 (0.07) days^{41,46}

Abbreviations: CI, confidence interval; DVT, deep vein thrombosis; GI, gastrointestinal; PY, person-years; VWD, von Willebrand disease.

Table 7 Disease Management in the Real-World Clinical Practice Setting

Treatment Indication	Main Product	Dose
Spontaneous bleeds (2 studies)	Haemate [®] P ⁹¹ Wilate ^{®87}	• 46.9 FVIII IU/kg for 1–21 days • 40.2 IU/kg for a mean of 5.3 ± 12.2 days
Long-term prophylaxis of overall VWD (6 studies)	Humate-P [®] , Fanhdi [®] and Alphanate ^{®95} Wilfactin ^{®92} Alphanate [®] , Fanhdi [®] or Humate-P ^{®97} Humate-P ^{®96} Fanhdi [®] and Alphanate ^{®98} FVIII ¹⁰¹	• Median (IQR) (VWF:RCo, IU/kg): ◦ Epistaxis: 48 (40–60)) ◦ GI bleeding: 60 (47–60) ◦ Joint bleeding: 40 (30–50) ◦ Other^a: 59 (17–70) ◦ Mixed^b: 42 (33–49) ◦ Abnormally heavy bleeding at menstruation: 39 (38–40) • Median of 43.5 VWF:RCo IU/kg per infusion, for patients on long-term prophylaxis (4239 IU/kg) • After the first bolus: 40 FVIII IU/kg twice a week for joint bleeding and every other day or 3×/week for GI bleeding • VWF:RCo: 40–80 IU/kg • FVIII:C 20–40 IU/kg • Median (range) long-term secondary prophylaxis dose: 42 (17–74) VWF:RCo IU/kg • Mean of 24 FVIII IU/kg 1–3×/week
Situational prophylaxis (11 studies)	Wilfactin ^{®92,111,112} Veyvondi [®] /Vonvendi ^{®113} Haemate [®] /Humate-P ^{®93,96,114,115} Fanhdi [®] and Alphanate ^{®98} Wilate ^{®116} Biostate ^{®88} Voncento ^{®117}	• Minor surgery: 54.0 VWF:RCo IU/kg • Major surgery: 44.5 VWF:RCo IU/kg • Dental procedures: 30 VWF:RCo IU/kg • Cesarean delivery: 27 VWF:RCo IU/kg • Vaginal delivery: 33–42 VWF:RCo IU/kg • Minor surgery: 35 VWF:RCo IU/kg pre-operatively and 34 VWF:RCo IU/kg maintenance • Major surgery: 36 VWF:RCo IU/kg pre-operatively and 26 VWF:RCo IU/kg maintenance • Minor surgery: initial bolus of 46–62 VWF:RCo IU/kg • Major surgery: initial bolus of 58–89 VWF:RCo IU/kg • Surgery: 40–80 VWF:RCo IU/kg; 20–40 FVIII:C IU/kg • Surgery: 80 VWF:RCo IU/kg at surgery and 40–50 VWF:RCo IU/kg post-operatively • Prior to delivery: 2000 IU • Surgery: preoperative dose: 48 VWF:RCo IU/kg; post-operative dose: 44 VWF:RCo IU/kg • Minor surgery: 42.5 IU/kg • Major surgery: 49 IU/kg • Dental procedures: 30 IU/kg • Minor surgery: 45 FVIII:C IU/kg for a median of 3 days • Major surgery: 51 FVIII:C IU/kg for a median of 7 days • Minor surgery: 38.9 ± 10.2 VWF:RCo IU/kg for a median of 1 day • Major surgery: 44.8 ± 19.0 VWF:RCo IU/kg for a median of 4.6 days

Notes: ^aOther: the primary indication occurred with low frequency. This category includes intracranial hemorrhage, hematomas in soft tissue, oral, dental extraction, scraped knees, ovarian cysts, or bleeding. ^bMixed: bleeding pattern was mixed with no primary indication.

Alphanate[®] (human FVIII/human VWF, Instituto Grifols, S.A., Barcelona, Spain); Biostate[®] (human FVIII/human VWF complex, CSL Behring (Australia) Pty Ltd, Broadmeadows, Australia); Fanhdi[®] (high-purity, double-inactivated plasma-derived FVIII/VWF concentrate, Instituto Grifols, S.A., Barcelona, Spain); Haemate[®]/Humate-P[®] (antihemophilic factor/VWF complex [human], CSL Behring GmbH, Marburg, Germany); Veyvondi[®]/Vonvendi[®] (recombinant VWF, Takeda Pharmaceuticals USA, Lexington, MA, USA); Voncento[®] (human FVIII/human VWF, CSL Behring GmbH, Marburg, Germany); Wilate[®] (human FVIII/human VWF, Octapharma AG, Lachen, Switzerland); Wilfactin[®] (human VWF, LFB-BIOMEDICAMENTS, Les Ulis, France).

Abbreviations: FVIII, factor VIII; FVIII:C, factor VIII concentration; GI, gastrointestinal; IQR, interquartile range; VWD, von Willebrand disease; VWF:RCo, von Willebrand factor:ristocetin cofactor.

number of patients with VWD, with less severe bleeding symptoms, than referral-based studies identifying symptomatic patients seeking care for VWD in specialist centers. The wide range in population-based estimates reflects variations in population size ($N = 182\text{--}4592$), as well as in timescale (study periods varied across a range of years from around 1985 to 2018 [exact timing not provided for study by Rodeghiero et al]).^{27–32} Definitions used to identify patients with VWD in the population-based studies also varied. Whereas all six population-based studies providing estimates for VWD overall^{27–32} included VWF levels with or without FVIII levels in the definition, the presence of bleeding symptoms was only required in three studies.^{28,30,31} Variations in referral-based estimates likely reflect several factors, including diagnostic facilities, physician awareness, subtype of VWD, and/or severity of bleeds.

Estimates of VWD prevalence also varied by disease type, age, and sex. Prevalence was lower in pediatric than in adult populations,¹² which could reflect the accumulation of non-fatal cases or increased diagnosis in adulthood. Type 3 VWD was the rarest reported (0.1–0.3 per 100,000), followed by type 2 VWD (0.8–2.5 per 100,000), and type 1 VWD (2.7–7.2 per 100,000).^{12,15,18} Reports suggest that more women than men are affected by VWD globally, despite VWD having an autosomal inheritance pattern.⁵ This may be because VWD is diagnosed more often in women due to female-specific hemostatic challenges such as HMB and intra-/postpartum bleeding. The studies reviewed suggest that HMB occurs frequently (>60%) in patients with VWD,^{23,31,34,42,44,54–58} while postpartum bleeding can occur in up to 13% of women with VWD.^{42,47,50,59–63}

This systematic review also suggests that there are important gaps in VWD diagnosis that could delay care and result in adverse health outcomes. Although the time from symptom development to diagnosis was poorly documented in the literature,^{38,52} the 3-year difference between median age at overt symptom onset and median age at diagnosis reported in this review³⁸ highlights the need for improved disease awareness for both patients and health care providers. According to international guidelines for VWD diagnosis, an accurate and timely diagnosis remains a major challenge for patients with VWD, with barriers identified as difficulties in distinguishing between normal and abnormal bleeding symptoms, limited clarity regarding an appropriate diagnostic approach, and limited access to specialized laboratory testing.¹⁰³ To facilitate VWD diagnosis, the multidisciplinary review panel recommended the use of bleeding-assessment tools in patients suspected of having VWD in a primary care setting to help identify patients who require laboratory testing, as well as specific diagnostic approaches for types 1 and 2 VWD.¹⁰³

Patients with VWD had a high burden of bleeding events even after VWD diagnosis or if receiving VWD treatment. Bleeding was reported in 72–94% of the patients with VWD,^{40,44,53} with mucocutaneous bleeds such as menorrhagia, GI bleeds, epistaxis, and oral bleeds being the most frequent.^{23,28,31,34,37,39,42–45,47,54–63,67,68,70–73,84} Life-threatening bleeding involving critical organs (eg, central nervous system^{34,37,44,55,69}) were also reported, although these were rare and studies have not found a difference in mortality between VWD and general populations.^{41,46,48,49,51} Only one study compared bleeding rates across all three VWD types.³⁴ In this retrospective Italian study ($N = 1234$), GI bleeding was more common in patients with types 2A or 3, central nervous system and joint bleeding events in those with type 3, and hematomas in patients with types 2N and 3, relative to other VWD types. However, the frequency of mucosal bleeding (epistaxis, HMB, and gum bleeding) did not differ significantly across VWD types. Lower bleeding rates were reported in studies that used medical records (vs those using questionnaires) or focused on specific patient populations, which suggests that bleeding events are not always reported or captured in medical records. Only one study used a validated bleeding assessment tool (the ISTH-BAT) completed by the physician to evaluate bleeding rates.⁵⁷ Bleeding assessment tools provide a standardized approach to assessment and can educate patients and physicians regarding bleeding symptoms and possible interventions.¹⁰³

The reported real-world treatment patterns show a range of treatment options with respect to VWD types and severity,^{92,95–97,99,100,102} with only a small proportion of patients with VWD reported to be receiving long-term VWF prophylaxis in a multinational VWD Prophylaxis Network study and in the Czech National Hemophilia Programme registry (0.7% and 1.6%, respectively).^{24,102} Long-term prophylaxis was administered most often in patients with type 3 VWD,^{92,95–97,99,100,102} and mostly for joint or GI bleeding.^{92,95–100,102} The current VWD management guidelines conditionally recommend long-term prophylaxis for patients with VWD and a history of severe and frequent bleeds.⁴ The guideline panel, however, acknowledged the low certainty of evidence for a net health benefit for long-term prophylaxis in this population. Thus, future research is needed to better understand bleeding outcomes and unmet needs of patients with VWD who may benefit from long-term prophylaxis.

Findings from the identified sources also suggest that VWD is associated with poorer HRQoL scores^{77,80,82} as well as higher HCRU^{83,84} compared with general populations. A variety of measurements were used across the eight studies evaluating HRQoL, including quality-of-life measures (SF-36 in adults, and ITQOL, CHQ-PF50, and CHQ-CF87 in children), and joint health measures (HJHS and HAL).^{39,40,67,73,77–82} In children, scores for physical functioning, emotional/behavioral functioning, and general health perceptions were lower than in children without VWD,⁷⁷ while in adults, both SF-36 PCS and MCS scores were lower than the general population.⁸⁰ All nine studies reporting HCRU were observational studies,^{36,38,40,41,46,57,69,83,84,90} mostly retrospective cohort studies. Although not covered by this review, decreased HRQoL and increased HCRU would be expected to have a substantial economic and societal impact. Studies evaluating VWD prophylaxis continue to demonstrate reductions in HCRU, such as hospitalizations,^{83,104} further supporting the overall benefit of long-term prophylaxis for eligible patients.

Limitations

Large variations in outcomes reported in this review reflect the heterogeneity of bleeding phenotype in VWD, as well as variability in study populations and research methodologies. Differences in disease awareness, diagnosis, and clinical management of VWD across the large geographic areas and different time periods covered by this review will also have contributed to the heterogeneity of the data.

Some relevant sources may not be captured in this review if they were not indexed in the electronic bibliographic databases used for this literature search, not classified under the MeSH or Emtree terms, or did not contain the keywords used in the search. To mitigate this limitation, the comprehensiveness of the search strategy was verified against articles known to be pertinent for the search. Keywords were reverse generated using a sample of articles deemed relevant for the review. Furthermore, some relevant sources may not have been captured in the systematic literature search, as some sources do not publish their studies in peer-reviewed journals. To mitigate this limitation, pragmatic searches of the gray literature as well as “snowballing” were conducted. Single-entry data extraction was employed for this systematic review. However, the completeness of the data was assessed by a separate reviewer.

Summary

This systematic review highlights the limited data available on the epidemiology of VWD, as well as the high disease burden and related impact on HRQoL and HCRU for patients with VWD. More real-world studies are needed to better understand the characteristics of VWD patient populations and help inform timely VWD diagnosis and care.

Data Sharing Statement

The datasets, including the template data extraction form and data extracted from the included studies, are available upon request from corresponding author, Ping Du.

Acknowledgments

The authors thank Teigna Arredondo-Bisono who provided support in the protocol development, data extraction, and analysis. Under the direction of the authors, medical writing support was provided by Joanne Vaughan, Excel Medical Affairs (Fairfield, CT, USA), and was funded by Takeda Development Center Americas, Inc., Lexington, MA, USA.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by Shire Human Genetic Therapies, Inc, a Takeda company, Lexington, MA. The sponsor was involved with the protocol development, analysis of identified sources and interpretation of findings, report development, and in the decision to submit the article for publication.

Disclosure

PD, GÖ, and ST are employees of Takeda Development Center Americas, Inc., and Takeda stockholders. AB and YM are employees of YOLARX Consultants and received fees from Shire Human Genetic Therapies, Inc., a Takeda company, for conducting this analysis. FTB is an employee of Takeda Pharmaceuticals International AG, and a Takeda stockholder. The authors report no other conflicts of interest in this work.

References

1. Gralnick HR, Williams S, McKeown L, et al. A monomeric von Willebrand factor fragment, Leu-504–Lys-728, inhibits von Willebrand factor interaction with glycoprotein Ib-IX [corrected]. *Proc Natl Acad Sci U S A*. 1992;89(17):7880–7884. doi:10.1073/pnas.89.17.7880
2. Sadler JE, Budde U, Eikenboom JC, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the subcommittee on von Willebrand Factor. *J Thromb Haemost*. 2006;4(10):2103–2114. doi:10.1111/j.1538-7836.2006.02146.x
3. Weiss HJ, Sussman II, Hoyer LW. Stabilization of factor VIII in plasma by the von Willebrand factor. Studies on posttransfusion and dissociated factor VIII and in patients with von Willebrand's disease. *J Clin Invest*. 1977;60(2):390–404. doi:10.1172/JCI108788
4. Connell NT, Flood VH, Brignardello-Petersen R, et al. ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease. *Blood Adv*. 2021;5(1):301–325. doi:10.1182/bloodadvances.2020003264
5. Leebeek FWG, Eikenboom JCJ. Von Willebrand's disease. *N Engl J Med*. 2016;375(21):2067–2080. doi:10.1056/NEJMra1601561
6. Bowman M, Hopman WM, Rapson D, Lillicrap D, James P. The prevalence of symptomatic von Willebrand disease in primary care practice. *J Thromb Haemost*. 2010;8(1):213–216. doi:10.1111/j.1538-7836.2009.03661.x
7. Cochrane group. Cochrane handbook for systematic reviews of interventions. Available from: <http://training.cochrane.org/handbook>. Accessed March 21, 2018.
8. Institute of Medicine (IOM). Standards for systematic reviews. Available from: <https://iom.nationalacademies.org/Reports/2011/Finding-What-Works-in-Health-Care-Standards-for-Systematic-Reviews/Standards.aspx>. Accessed March 21, 2018.
9. Orphanet. Von Willebrand disease. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=EN&Expert=903. Accessed May 31, 2022.
10. World Federation of Hemophilia. Report on the annual global survey; 2019. Available from: <http://www1.wfh.org/publications/files/pdf-1806.pdf>. Accessed May 31, 2022.
11. Mannucci PM, Bloom AL, Larrieu MJ, Nilsson IM, West RR. Atherosclerosis and von Willebrand factor. I. Prevalence of severe von Willebrand's disease in Western Europe and Israel. *Br J Haematol*. 1984;57(1):163–169. doi:10.1111/j.1365-2141.1984.tb02876.x
12. Abbonizio F, Hassan HJ, Riccioni R, Santagostino E, Arcieri R, Giampaolo A. New data from the Italian National Register of congenital coagulopathies, 2016 annual survey. *Blood Transfus*. 2020;18(1):58–66. doi:10.2450/2019.0211-18
13. FranceCoag. National statistics: von Willebrand disease. Available from: https://www.francecoag.org/SiteWebPublic/public/stats/stats_page.jsp?stat1=on&stat4=on. Accessed March 31, 2021.
14. Giampaolo A, Abbonizio F, Arcieri R. Italian national registry of congenital coagulopathies. *Blood Transfus*. 2020;18(3):s30.
15. United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO). UKHCDO annual report 2020 & bleeding disorder statistics for the financial year 2019/2020; 2020.
16. Giampaolo A, Abbonizio F, Arcieri R, Hassan HJ. Italian registry of congenital bleeding disorders. *J Clin Med*. 2017;6(3):34. doi:10.3390/jcm6030034
17. Korea Hemophilia Foundation (KHF). Current Status. Available from: <http://www.kohem.org/eng/load.asp?subPage=310>. Accessed December 31, 2020.
18. National Blood Authority of Australia. Australian bleeding disorders registry: annual report 2018-19; 2019. Available from: <https://www.blood.gov.au/system/files/ABDR-Annual-Report-2018-19-FINAL.pdf>. Accessed November 23, 2021.
19. Tatsunami S, Kuwabara R, Akita M, Mimaya J, Shirahata A, Taki M. Number of patients with coagulation disorders in Japan's 2010 annual report from the research committee for the national surveillance on coagulation disorders in Japan. *Haemophilia*. 2012;18(3):84.
20. Yoon HS, Han Y, Kim YJ, et al. Epidemiology of congenital bleeding disorders: a nationwide population-based Korean study. *J Korean Med Sci*. 2020;35(39):e350. doi:10.3346/jkms.2020.35.e350
21. Qu Y, Nie X, Yang Z, et al. The prevalence of hemophilia in mainland China: a systematic review and meta-analysis. *Southeast Asian J Trop Med Public Health*. 2014;45(2):455–466.
22. Alkaabi S, Alzadjali A, Wasifuddin M, et al. Spectrum, clinical characteristics and outcome of von Willebrand disease in Oman. *Haemophilia*. 2020;26(Suppl 2):142.
23. Mansouritorghabeh H, Manavifar L, Banihashem A, et al. An investigation of the spectrum of common and rare inherited coagulation disorders in north-eastern Iran. *Blood Transfus*. 2013;11(2):233–240. doi:10.2450/2012.0023-12
24. Blatný J, Ovesná P. The status of care for persons with von Willebrand disease registered within CNHP registry annual report; 2019. Available from: https://www.cnhp.cz/res/file/vysledky/cnhp_2019_vwd_print.pdf. Accessed August 19, 2022.
25. Centers for Disease Control and Prevention. HTC population profile patient characteristics. Available from: <https://www.cdc.gov/ncbddd/hemophilia/communitycounts/data-reports/2020-9/table-1-patient-characteristics-by-calendar.html>. Accessed August 19, 2022.

26. The World Bank. Population, Total – Australia; 2019. Available from: <https://data.worldbank.org/indicator/SP.POP.TOTL?locations=AU>. Accessed November 23, 2021.
27. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood*. 1987;69(2):454–459.
28. Sap F, Kavakli T, Kavakli K, Dizdärer C. The prevalence of von Willebrand disease and significance of in vitro bleeding time (PFA-100) in von Willebrand disease screening in the Izmir region. *Turk J Haematol*. 2013;30(1):40–47. doi:10.4274/tjh.2011.0020
29. Abu-Douleh E, Al-Numair N, Albanyan A, Alsuliman A, Bayoumi N, Owaidah T. Prevalence of von Willebrand disease among university students in Riyadh, Saudi Arabia. *J Appl Hematol*. 2018;9(4):136–139. doi:10.4103/joah.joah_63_18
30. Bowman M, Hopman WM, Rapson D, Lillicrap D, Silva M, James P. A prospective evaluation of the prevalence of symptomatic von Willebrand disease (VWD) in a pediatric primary care population. *Pediatr Blood Cancer*. 2010;55(1):171–173. doi:10.1002/pbc.22429
31. Werner EJ, Broxson EH, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: a multiethnic study. *J Pediatr*. 1993;123(6):893–898. doi:10.1016/s0022-3476(05)80384-1
32. Shonde-Adebola KB, Shokunbi WA, Adebola MB. Prevalence of von Willebrand disease among Nigerian youths in Ibadan South-Western Nigeria. *OALib*. 2021;8:e7789. doi:10.4236/oalib.1107789
33. Orphanet. Von Willebrand disease type 3. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=en&Expert=166096. Accessed April 19, 2021.
34. Federici AB, Bucciarelli P, Castaman G, et al. Management of inherited von Willebrand disease in Italy: results from the retrospective study on 1234 patients. *Semin Thromb Hemost*. 2011;37(5):511–521. doi:10.1055/s-0031-1281037
35. Federici AB, Bucciarelli P, Castaman G, et al. The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand disease. *Blood*. 2014;123(26):4037–4044. doi:10.1182/blood-2014-02-557264
36. Holm E, Osoli M, Steen Carlsson K, Berntorp E. Cardiovascular disease-related hospitalization and mortality among persons with von Willebrand disease: a nationwide register study in Sweden. *Haemophilia*. 2019;25(1):109–115. doi:10.1111/hae.13642
37. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al. von Willebrand disease and aging: an evolving phenotype. *J Thromb Haemost*. 2014;12(7):1066–1075. doi:10.1111/jth.12586
38. Siboni SM, Biguzzi E, Caiani V, Mistretta C, Bucciarelli P, Peyvandi F. Baseline factor VIII plasma levels and age at first bleeding in patients with severe forms of von Willebrand disease. *Haemophilia*. 2016;22(4):564–569. doi:10.1111/hae.12900
39. van Galen KPM, Sanders YV, Vojinovic U, et al. Joint bleeds in von Willebrand disease patients have significant impact on quality of life and joint integrity: a cross-sectional study. *Haemophilia*. 2015;21(3):e185–e192. doi:10.1111/hae.12670
40. Abe K, Dupervil B, O'Brien SH, et al. Higher rates of bleeding and use of treatment products among young boys compared to girls with von Willebrand disease. *Am J Hematol*. 2020;95(1):10–17. doi:10.1002/ajh.25656
41. Apostolova MH, Seaman CD, Comer DM, Yabes JG, Ragni MV. Prevalence and risk factors associated with hypertension in von Willebrand disease. *Clin Appl Thromb Hemost*. 2018;24(1):93–99. doi:10.1177/1076029616670258
42. Kumar S, Kishore R, Gupta V, Jain M, Shukla J. Prevalence and spectrum of von Willebrand disease in Eastern Uttar Pradesh. *Indian J Pathol Microbiol*. 2010;53(3):486–489. doi:10.4103/0377-4929.68287
43. Mokhtar GM, Tantawy AA, Adly AA, Telbany MA, El Arab SE, Ismail M. A longitudinal prospective study of bleeding diathesis in Egyptian pediatric patients: single-center experience. *Blood Coagul Fibrinolysis*. 2012;23(5):411–418. doi:10.1097/MBC.0b013e3283540bf8
44. Öner N, Gürsel T, Kaya Z, et al. Inherited coagulation disorders in Turkish children: a retrospective, single-center cohort study. *Transfus Apher Sci*. 2020;59(3):102728. doi:10.1016/j.transci.2020.102728
45. Sahoo T, Naseem S, Ahluwalia J, Marwaha RK, Trehan A, Bansal D. Inherited bleeding disorders in North Indian children: 14 years' experience from a tertiary care center. *Indian J Hematol Blood Transfus*. 2020;36(2):330–336. doi:10.1007/s12288-019-01233-3
46. Seaman CD, Yabes J, Comer DM, Ragni MV. Does deficiency of von Willebrand factor protect against cardiovascular disease? Analysis of a national discharge register. *J Thromb Haemost*. 2015;13(11):1999–2003. doi:10.1111/jth.13142
47. Trasi S, Shetty S, Ghosh K, Mohanty D. Prevalence and spectrum of von Willebrand disease from western India. *Indian J Med Res*. 2005;121(5):653–658.
48. Qureshi W, Hassan S, Dabak V, Kuriakose P. Thrombosis in VonWillebrand disease. *Thromb Res*. 2012;130(5):e255–e258. doi:10.1016/j.thromres.2012.09.005
49. Henriksson KM, Astermark J, Nilsson J-A, Baghaei F, Holmstrom M, Berntorp E. Survival among 5118 patients with bleeding disorders compared to matched controls in Sweden: results from a cohort register study. *J Thromb Haemost*. 2011;9(Suppl 2):222.
50. Malec LM, Moore CG, Yabes J, Li J, Ragni MV. Postpartum haemorrhage in women with von Willebrand disease: an observational study of the Pennsylvania Health Care Cost Containment Council (PHC4) database. *Haemophilia*. 2015;21(5):e442–e445. doi:10.1111/hae.12739
51. Mittal V, Ahuja S, Acharya C, Palabindala V, Milner C. Obstetric and bleeding complications in pregnancy with von Willebrand disease: results from nationwide inpatient sample database 2003–2011. *Blood*. 2017;130(1):3373.
52. Sidonio RF, Haley KM, Fallaize D. Impact of diagnosis of von Willebrand disease on patient outcomes: analysis of medical insurance claims data. *Haemophilia*. 2017;23(5):743–749. doi:10.1111/hae.13292
53. Tosetto A, Rodeghiero F, Castaman G, et al. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost*. 2006;4(4):766–773. doi:10.1111/j.1538-7836.2006.01847.x
54. Chen YC, Yang L, Cheng SN, Hu SH, Chao TY. von Willebrand disease: a clinical and laboratory study of sixty-five patients. *Ann Hematol*. 2011;90(10):1183–1190. doi:10.1007/s00277-011-1266-4
55. de Wee EM, Sanders YV, Mauser-Bunschoten EP, et al. Determinants of bleeding phenotype in adult patients with moderate or severe von Willebrand disease. *Thromb Haemost*. 2012;108(4):683–692. doi:10.1160/TH12-04-0244
56. Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol*. 2000;111(4):1236–1239. doi:10.1046/j.1365-2141.2000.02507.x
57. Sanders YV, Fijnvandraat K, Boender J, et al. Bleeding spectrum in children with moderate or severe von Willebrand disease: relevance of pediatric-specific bleeding. *Am J Hematol*. 2015;90(12):1142–1148. doi:10.1002/ajh.24195
58. Tosetto A, Badiee Z, Baghaipour MR, et al. Bleeding symptoms in patients diagnosed as type 3 von Willebrand disease: results from 3WINTERS-IPS, an international and collaborative cross-sectional study. *J Thromb Haemost*. 2020;18(9):2145–2154. doi:10.1111/jth.14886
59. Hassan S, Qureshi W, Donthireddy V, Kuriakose P. Congenital von Willebrand's disease and clinical hypothyroidism. *Haemophilia*. 2013;19(2):242–245. doi:10.1111/hae.12065

60. O'Brien SH, Stanek JR, Kaur D, McCracken K, Vesely SK. Laboratory monitoring during pregnancy and post-partum hemorrhage in women with von Willebrand disease. *J Thromb Haemost*. 2020;18(3):604–608. doi:10.1111/jth.14696
61. Xu Z, Dargaud Y, Chamouard V, et al. A single-centre study of management of pregnant women with von Willebrand disease. *Haemophilia*. 2019;25(1):e54–e57. doi:10.1111/hae.13653
62. Majluf-Cruz A, Velez-Ruelas MA, Gonzalez-Avila AI, et al. von Willebrand's disease in Mexico: a pilot study. *Haemophilia*. 2013;19(2):231–235. doi:10.1111/hae.12016
63. Wilson E, Hanna F, Hyppa A, et al. Assessment of von Willebrand disease and pregnancy outcomes at regional Australian hospitals. *Eur J Haematol*. 2021;106(4):456–466. doi:10.1111/ejh.13562
64. Skeith L, Rydz N, O'Beirne M, Goodyear D, Li H, Poon MC. Pregnancy loss in women with von Willebrand disease: a single-center pilot study. *Blood Coagul Fibrinolysis*. 2017;28(5):393–397. doi:10.1097/MBC.0000000000000620
65. Sladic M, Smrkolj S. The impact of von Willebrand's disease on pregnancy, delivery and postpartum period: a population-based study. *Eur J Obstet Gynecol Reprod Biol*. 2019;234:E155.
66. Yousuf S, Cohen AJ, Eris E, Astsaturov A. A single institutional study on pregnancy outcomes in patients with von Willebrand disease. *Blood*. 2017;130(Suppl 1):1086. doi:10.1182/blood.V130.Suppl_1.1086.1086
67. Rae C, Furlong W, Horsman J, et al. Bleeding disorders, menorrhagia and iron deficiency: impacts on health-related quality of life. *Haemophilia*. 2013;19(3):385–391. doi:10.1111/hae.12014
68. Kaur D, Vesely SK, Koch T, O'Brien SH. Quality and consequences of laboratory monitoring for von Willebrand disease during pregnancy. *Am J Hematol*. 2018;93(9):E31–E32.
69. Lu M, Oladapo A, Wu Y, Farahbakhshian S, Ewenstein B. Economic burden of major bleeding events in commercially insured patients with von Willebrand disease based on claims data from the United States. *J Manag Care Spec Pharm*. 2021;27(2):175–185. doi:10.18553/jmcp.2020.20327
70. Tsagianni A, Comer DM, Yabes JG, Ragni MV. Von Willebrand disease and gastrointestinal bleeding: a national inpatient sample study. *Thromb Res*. 2019;178:119–123. doi:10.1016/j.thromres.2019.04.017
71. Haley KM, Sidonio RF, Abraham S, Cheng D, Recht M, Kulkarni R. A cross-sectional study of women and girls with congenital bleeding disorders: the American Thrombosis and Hemostasis Network cohort. *J Womens Health*. 2020;29(5):670–676. doi:10.1089/jwh.2019.7930
72. van Deukeren D, Mauser-Bunschoten EP, Schutgens REG, et al. The prevalence and burden of hand and wrist bleeds in von Willebrand disease. *Haemophilia*. 2019;25(1):e35–e38. doi:10.1111/hae.13632
73. van Galen KPM, Meijer K, Vogely HC, et al. Joint surgery in von Willebrand disease: a multicentre cross-sectional study. *Haemophilia*. 2016;22(2):256–262. doi:10.1111/hae.12834
74. Biss TT, Blanchette VS, Clark DS, et al. Quantitation of bleeding symptoms in children with von Willebrand disease: use of a standardized pediatric bleeding questionnaire. *J Thromb Haemost*. 2010;8(5):950–956. doi:10.1111/j.1538-7836.2010.03796.x
75. Atiq F, Meijer K, Eikenboom J, et al. Comorbidities associated with higher von Willebrand factor (VWF) levels may explain the age-related increase of VWF in von Willebrand disease. *Br J Haematol*. 2018;182(1):93–105. doi:10.1111/bjh.15277
76. Franchini M, Di Perna C, Santoro C, et al. Cancers in patients with von Willebrand disease: a survey from the Italian Association of Haemophilia Centres. *Semin Thromb Hemost*. 2016;42(1):36–41. doi:10.1055/s-0035-1564844
77. de Wee EM, Fijnvandraat K, de Goede-Bolder A, et al. Impact of von Willebrand disease on health-related quality of life in a pediatric population. *J Thromb Haemost*. 2011;9(3):502–509. doi:10.1111/j.1538-7836.2010.04175.x
78. McLaughlin JM, Munn JE, Anderson TL, Lambing A, Tortella B, Witkop ML. Predictors of quality of life among adolescents and young adults with a bleeding disorder. *Health Qual Life Outcomes*. 2017;15(1):67. doi:10.1186/s12955-017-0643-7
79. van Galen KPM, de Kleijn P, Foppen W, et al. Long-term impact of joint bleeds in von Willebrand disease: a nested case-control study. *Haematologica*. 2017;102(9):1486–1493. doi:10.3324/haematol.2017.168617
80. Xu Y, Deforest M, Grabell J, Hopman W, James P. Relative contributions of bleeding scores and iron status on health-related quality of life in von Willebrand disease: a cross-sectional study. *Haemophilia*. 2017;23(1):115–121. doi:10.1111/hae.13062
81. van Galen KPM, Timmer M, de Kleijn P, et al. Long-term outcome after joint bleeds in von Willebrand disease compared to haemophilia A: a post hoc analysis. *Thromb Haemost*. 2018;118(10):1690–1700. doi:10.1055/s-0038-1670704
82. Govorov I, Ekelund L, Chaireti R, et al. Heavy menstrual bleeding and health-associated quality of life in women with von Willebrand's disease. *Exp Ther Med*. 2016;11(5):1923–1929. doi:10.3892/etm.2016.3144
83. Holm E, Carlsson KS, Lovdahl S, Lail AE, Abshire TC, Berntorp E. Bleeding-related hospitalization in patients with von Willebrand disease and the impact of prophylaxis: results from national registers in Sweden compared with normal controls and participants in the von Willebrand Disease Prophylaxis Network. *Haemophilia*. 2018;24(4):628–633. doi:10.1111/hae.13473
84. Oladapo A, Wu Y, Lu M, Farahbakhshian S, Ewenstein B. Estimation of the economic burden associated with major surgery due to von Willebrand disease based on claims data from the USA. *Blood*. 2019;134(1):4692.
85. Boban A, Lambert CM, Hermans C. VWF-FVIII concentrates in the treatment of inherited von Willebrand disease: a single-centre retrospective study. *Haemophilia*. 2019;25(4):e300–e303. doi:10.1111/hae.13779
86. De Wee EM, Knol HM, Mauser-Bunschoten EP, et al. Gynaecological and obstetric bleeding in moderate and severe von Willebrand disease. *Thromb Haemost*. 2011;106(5):885–892. doi:10.1160/TH11-03-0180
87. Batty P, Chen YH, Bowles L, Hart DP, Platten S, Pasi KJ. Safety and efficacy of a von Willebrand factor/factor VIII concentrate (Wilate(R)): a single centre experience. *Haemophilia*. 2014;20(6):846–853. doi:10.1111/hae.12496
88. Howman R, Barnes C, Curtin J, et al. The clinical efficacy and safety of the FVIII/VWF concentrate, BIOSTATE(R), in children with von Willebrand disorder: a multi-centre retrospective review. *Haemophilia*. 2011;17(3):463–469. doi:10.1111/j.1365-2516.2010.02445.x
89. Khair K, Batty P, Riat R, et al. Wilate use in 47 children with von Willebrand disease: the North London paediatric haemophilia network experience. *Haemophilia*. 2015;21(1):e44–e50. doi:10.1111/hae.12497
90. Livesey K, Yealy DM, Li J, Moore CG, Ragni MV. Von Willebrand disease in the emergency department. *Haemophilia*. 2016;22(2):263–267. doi:10.1111/hae.12841
91. Miesbach W, Krekeler S, Wolf Z, Seifried E. Clinical use of Haemate(R) P in von Willebrand disease: a 25-year retrospective observational study. *Thromb Res*. 2015;135(3):479–484. doi:10.1016/j.thromres.2014.12.017

92. Goudemand J, Briday F, Claeysens S, et al. Management of von Willebrand disease with a factor VIII-poor von Willebrand factor concentrate: results from a prospective observational post-marketing study. *J Thromb Haemost*. 2020;18(8):1922–1933. doi:10.1111/jth.14928
93. Govorov I, Lofgren S, Chairati R, Holmstrom M, Bremme K, Mints M. Postpartum hemorrhage in women with von Willebrand disease - a retrospective observational study. *PLoS One*. 2016;11(10):e0164683. doi:10.1371/journal.pone.0164683
94. James AH, Konkole BA, Kouides P, et al. Postpartum von Willebrand factor levels in women with and without von Willebrand disease and implications for prophylaxis. *Haemophilia*. 2015;21(1):81–87. doi:10.1111/hae.12568
95. Abshire TC, Federici AB, Alvarez MT, et al. Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). *Haemophilia*. 2013;19(1):76–81. doi:10.1111/j.1365-2516.2012.02916.x
96. Castaman G, Coppola A, Zanon E, et al. Efficacy and safety during formulation switch of a pasteurized VWF/FVIII concentrate: results from an Italian prospective observational study in patients with von Willebrand disease. *Haemophilia*. 2013;19(1):82–88. doi:10.1111/hae.12005
97. Federici AB, Gianniello F, Mannucci PM. Secondary long-term prophylaxis in von Willebrand disease: an Italian cohort study. *Hematology Meeting Reports*. 2005;1:6. doi:10.4081/hmr.v1i6.270
98. Federici AB, Barillari G, Zanon E, et al. Efficacy and safety of highly purified, doubly virus-inactivated VWF/FVIII concentrates in inherited von Willebrand's disease: results of an Italian cohort study on 120 patients characterized by bleeding severity score. *Haemophilia*. 2010;16(1):101–110. doi:10.1111/j.1365-2516.2009.02088.x
99. Holm E, Abshire TC, Bowen J, et al. Changes in bleeding patterns in von Willebrand disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network. *Blood Coagul Fibrinolysis*. 2015;26(4):383–388. doi:10.1097/MBC.0000000000000257
100. Halimeh S, Krumpel A, Rott H, et al. Long-term secondary prophylaxis in children, adolescents and young adults with von Willebrand disease. Results of a cohort study. *Thromb Haemost*. 2011;105(4):597–604. doi:10.1160/TH10-09-0616
101. Bernthorpe E. Prophylaxis and treatment of bleeding complications in von Willebrand disease type 3. *Semin Thromb Hemost*. 2006;32(6):621–625. doi:10.1055/s-2006-949667
102. Bernthorpe E, Abshire T. The von Willebrand disease prophylaxis network: exploring a treatment concept. *J Thromb Haemost*. 2006;4(11):2511–2512. doi:10.1111/j.1538-7836.2006.02179.x
103. James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. *Blood Adv*. 2021;5(1):280–300. doi:10.1182/bloodadvances.2020003265
104. Schinco P, Cultrera D, Valeri F, et al. Cost-consequence analysis of long-term prophylaxis in the treatment of von Willebrand disease in the Italian context. *Clinicoecon Outcomes Res*. 2015;7:17–25. doi:10.2147/CEOR.S71892
105. Population Estimate as of January 1, 2021. Institut national de statistique et des études économiques (INSEE); 2021. Available from: <https://www.insee.fr/fr/statistiques/1893198>. Accessed November 23, 2021.
106. Weiss HJ, Ball AP, Mannucci PM. Incidence of severe von Willebrand's disease. *N Engl J Med*. 1982;307(2):127. doi:10.1056/NEJM198207083070222
107. The World Bank. Population, total - Japan. Available from: <https://data.worldbank.org/indicator/SP.POP.TOTL?locations=JP>. Accessed November 23, 2021.
108. The World Bank. Population, Total - Korea, Republic. Available from: <https://data.worldbank.org/indicator/SP.POP.TOTL?locations=KR>. Accessed April 27, 2022.
109. Lu M, Oladapo A, Wu Y, Farahbakhshian S, Ewenstein B. Prevalence and burden of major bleeding events in patients with von Willebrand disease based on claims data from the USA. *Blood*. 2018;132(Suppl 1):2222.
110. Turan O. Gynaecological problem and their management in women with moderate and severe von Willebrand disease (VWD). *Haemophilia*. 2020;26(Suppl 4):139–140.
111. Borel-Derlon A, Goudemand J, Boyer-Neumann C, et al. Gynecological & obstetrical events from a French post-marketing survey of a von Willebrand factor concentrate with a low factor VIII content. *J Thromb Haemost*. 2011;9(Suppl 2):667–668.
112. Boyer-Neumann C, Borel-Derlon A, Goudemand J, et al. Management of childbirth in Von Willebrand Disease (VWD) women: results from studies with a von Willebrand factor product with a low content of Factor VIII. *Hamostaseologie*. 2013;33(1):A42.
113. Desprez D, Drillaud N, Flaujac C, et al. Efficacy and safety of a recombinant Von Willebrand Factor treatment in patients with inherited Von Willebrand Disease requiring surgical procedures. *Haemophilia*. 2021;27(2):270–276. doi:10.1111/hae.14242
114. Auerswald G, Bade A, Haubold K, Overberg D, Masurat S, Moorthi C. No inhibitor development after continuous infusion of factor concentrates in subjects with bleeding disorders undergoing surgery: a prospective study. *Haemophilia*. 2013;19(3):438–444. doi:10.1111/hae.12075
115. Seaman CD, Ragni MV. Periprocedural management of von Willebrand disease: an institutional experience. *Haemophilia*. 2019;25(3):e199–e203. doi:10.1111/hae.13718
116. O'Connell M, Singleton E, Byrne MB, Ryan K, White B, O'Donnell J. Efficacy and safety of Wilate following an en masse switch for patients with inherited von Willebrand disease. *J Thromb Haemost*. 2013;11(2):550.
117. Proulle V, Trossaert M, Rugeri L, et al. Efficacy and safety of hFVIII/VWF concentrate in a large cohort of patients with von Willebrand disease (VWD) requiring surgical procedures: the French experience. *Res Pract Thromb Haemost*. 2019;3:389–390.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Prolonged Exposure to Caplacizumab as Rescue Therapy in Refractory Immune Thrombotic Thrombocytopenic Purpura

George M Rodgers, Misa Berndt, Megan Fonteno & Jeffrey A Gilreath

To cite this article: George M Rodgers, Misa Berndt, Megan Fonteno & Jeffrey A Gilreath (2023) Prolonged Exposure to Caplacizumab as Rescue Therapy in Refractory Immune Thrombotic Thrombocytopenic Purpura, Journal of Blood Medicine, , 209-211, DOI: [10.2147/JBM.S395248](https://doi.org/10.2147/JBM.S395248)

To link to this article: <https://doi.org/10.2147/JBM.S395248>



© 2023 Rodgers et al.



Published online: 02 Mar 2023.



Submit your article to this journal



Article views: 186



View related articles



View Crossmark data

Prolonged Exposure to Caplacizumab as Rescue Therapy in Refractory Immune Thrombotic Thrombocytopenic Purpura

George M Rodgers ¹, Misa Berndt², Megan Fonteno³, Jeffrey A Gilreath ^{2,4}

¹Division of Hematology and Hematologic Malignancies Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT, USA; ²Sugarhouse Hematology, University of Utah, Salt Lake City, UT, USA; ³Clinical Pharmacy, MD Anderson Cancer Institute, Houston, TX, USA; ⁴Department of Pharmacotherapy, University of Utah College of Pharmacy, Salt Lake City, UT, USA

Correspondence: George M Rodgers, Division of Hematology and Hematologic Malignancies, University of Utah Medical Center, Salt Lake City, UT, 84132, USA, Tel +1 801-213-2076, Email George.rodgers@hsc.utah.edu

Abstract: We describe a case of refractory thrombotic thrombocytopenic purpura (7 lines of therapy) in which caplacizumab was used over a 6-month period as rescue therapy. Caplacizumab maintained the patient in clinical remission until successful immunosuppression was achieved resulting in normal ADAMTS13 levels. This case illustrates the utility of caplacizumab therapy in treating refractory TTP.

Keywords: thrombotic thrombocytopenic purpura, caplac izumab, ADAMTS13, von Willebrand factor

Introduction

Caplacizumab is a humanized immunoglobulin fragment targeting the A1 domain (platelet binding site) of von Willebrand factor. The drug is recommended to be given as a daily subcutaneous injection to prevent platelet thrombosis and organ dysfunction associated with immune thrombotic thrombocytopenic purpura (TTP).^{1,2} The International Society of Thrombosis and Haemostasis gives caplacizumab a “conditional recommendation” to treat TTP because clinical trial data with the drug did not clearly demonstrate a mortality advantage, and the high cost of the drug may be a barrier to its use.³ Current prescribing information for caplacizumab recommends 30 days of treatment, with an additional 28 days suggested for persistent disease.

We describe a patient with immune TTP, refractory to 7 lines of therapy, who despite 6 months of severe ADAMTS13 deficiency (<5% of normal activity levels) achieved and maintained a stable normalized platelet count using outpatient caplacizumab until his ultimate response to bortezomib therapy.

Case Report

In 2004, a 47-year-old male with multiple auto-immune conditions (ulcerative colitis, Graves disease, diabetes) was diagnosed with immune TTP and treated with steroids, plasma exchange (PLEX), vincristine, splenectomy, and rituximab. He was in remission until 2019, at which point prednisone, rituximab, and PLEX led to a second remission lasting until 2021, when at age 63, he presented with recurrent TTP; ADAMTS13 activity was undetectable, and he had a positive ADAMTS13 inhibitor test at >8 Bethesda units. His clinical presentation was acute stroke, and there was exacerbation of his chronic kidney disease. During this time, he received prednisone (1 mg/kg daily), PLEX, (1 plasma volume daily for 8 days), and caplacizumab. Rituximab therapy was delayed so that the patient could receive and respond to COVID19 immunization. He was continued on caplacizumab because of persistently undetectable ADAMTS13 activity levels. During this time, ADAMTS13 inhibitor levels were consistently greater than 8 Bethesda units.

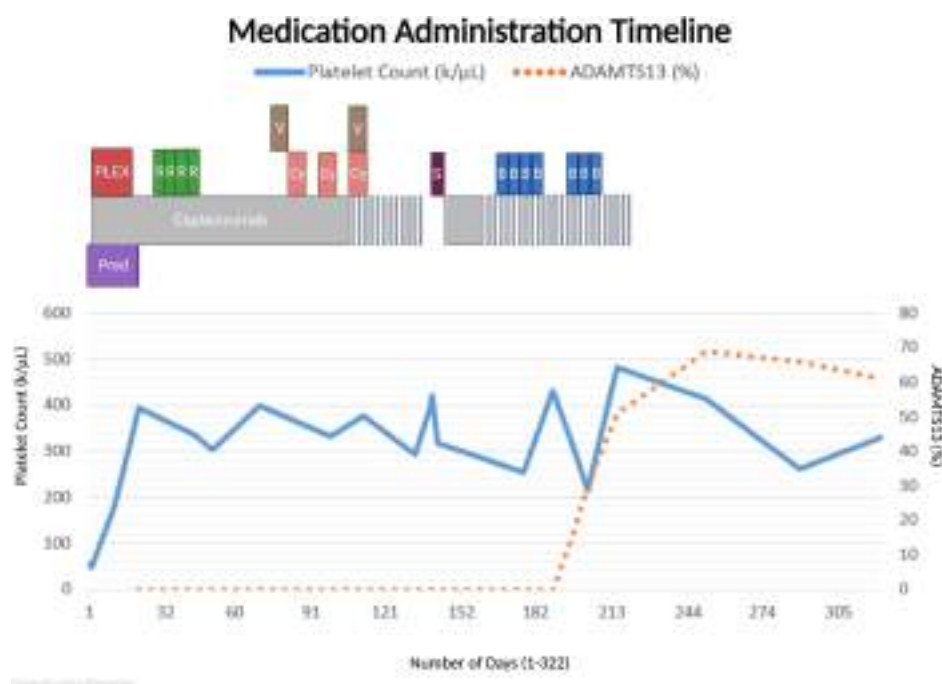


Figure 1 Medication timeline and response of platelet count and ADAMTS13 levels. The graph summarizes medications administered during the refractory TTP episode. Initial treatment was prednisone (day 1), PLEX and caplacizumab (day 2). Prednisone 1 mg/kg PO daily was continued d1-d10; PLEX daily d2-d9; and caplacizumab 11 mg SC daily d2-d15. By d20 the platelet count was 400,000/ μ L and the ADAMTS13 activity remained <5%. Rituximab 375 mg/ m^2 IV weekly for 4 doses was given d28-d50. Vincristine 2 mg IV was given on d69 and d117. Cyclophosphamide was given 400 mg/ m^2 IV every 3 weeks for 3 doses between d75-d117 before accessory spleen removal on d140. Caplacizumab was continued every other day from d117-d135 due to drug procurement issues. Daily caplacizumab was resumed post-splenectomy from d143-d162. Platelets remained stable during this time; however, ADAMTS13 activity remained undetectable. To conserve caplacizumab drug supply, alternate-day dosing was again used between d164-d210. Bortezomib 1.3 mg/ m^2 SC was administered for refractory disease on d166, d169, d173, d177, d195, d198, and d202. ADAMTS13 activity increased after the fourth bortezomib dose. After stopping all medications, ADAMTS13 activity rose into the normal range.

Abbreviations: PLEX, plasma exchange; V, vincristine; Cy, cyclophosphamide; S, accessory spleen removal; B, bortezomib; R, rituximab.

Figure 1 summarizes the patient's treatments, platelet counts, and ADAMTS13 activity levels during this episode of TTP recurrence. Treatments included rituximab, vincristine, IV cyclophosphamide (400 mg/ m^2 every 3 weeks for 3 doses),⁴ accessory spleen removal, and bortezomib (1.3 mg/ m^2 for 7 doses).⁵ Steroids were not repeated due to poorly-controlled diabetes. Otherwise, treatment toxicity was minimal except for bortezomib-associated diarrhea and dehydration.

ADAMTS13 activity became detectable, and ADAMTS13 inhibitor began to decrease starting one month after bortezomib therapy was started. Caplacizumab was discontinued; he received ~6 months of caplacizumab therapy while awaiting response to immunosuppression. He did not experience significant bleeding events on caplacizumab. During the last 2 months of caplacizumab therapy, dosage was changed from daily to every other day (Figure 1). Platelet counts remained normal on the less-frequent dosing schedule. Laboratory monitoring of von Willebrand factor activity was not done during his treatment. At this time, the patient has been in complete remission for 7 months. Most recent ADAMTS13 activity was 80%.

Discussion

The patient ultimately achieved remission using bortezomib. A recent review identified 32 patients reported in the literature and at a single institution who received bortezomib therapy for refractory TTP.⁶ ~70% of patients had a complete response; median time to relapse was 7 months, and 6% of patients had adverse events.⁶

Caplacizumab induces acquired von Willebrand disease, and consequently, a potential bleeding risk in TTP patients. A recent literature review of the drug's safety profile raised concerns about major bleeding to argue against routine use of caplacizumab to treat immune TTP.⁷ Our patient received ~ 6 months of daily or alternate-day caplacizumab and

experienced no major bleeding events. Future clinical trials should address this issue and determine whether caplacizumab's efficacy benefits outweigh its bleeding risks.

Recent data from a European registry of TTP cases treated with or without caplacizumab indicate that all treatment-related outcomes, including mortality were improved with caplacizumab therapy.⁸ The above case report suggests that it is feasible to use caplacizumab as rescue therapy for refractory TTP patients. Caplacizumab drug costs can be reduced by utilizing an alternate-day dosing regimen.⁹

Ethical Statement

This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from the patient in this study for publication of this case report. Institutional approval was not required for this case report.

Funding

No funding was received for this study.

Disclosure

GMR is a consultant for and reports personal fees from Sanofi. MB declares that she has no conflicts of interest in this work. MF declares that she has no conflicts of interest in this work. JAG declares that he has no conflicts of interest in this work.

References

1. Duggan S. Caplacizumab: first global approval. *Drugs*. 2018;78:1639–1642. doi:10.1007/s40265-018-0989-0
2. Scully M, Cataland SR, Peyvandi F, et al. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. *N Engl J Med*. 2019;380:335–346. doi:10.1056/NEJMoa1806311
3. Zheng XL, Vesely SK, Cataland SR, et al. ISTH guidelines for treatment of thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2020;18:2496–2502. doi:10.1111/jth.15010
4. Abou-Ismaïl MY, Arafah Y, Fu P, et al. Outcomes of immune thrombotic thrombocytopenic purpura (iTTP) with upfront cyclophosphamide vs rituximab. *Front Med*. 2020;7:588526. doi:10.3389/fmed.2020.588526
5. Shortt J, Oh DH, Opat SS. ADAMTS13 antibody depletion by bortezomib in thrombotic thrombocytopenic purpura. *N Engl J Med*. 2013;368:90–92. doi:10.1056/NEJMc1213206
6. Lee NCJ, Yates S, Rambally S, et al. Bortezomib in relapsed/refractory autoimmune-mediated thrombotic thrombocytopenic purpura. A single-center case series and systematic review. American Society of Hematology Annual Meeting. Poster #1135; 2022.
7. Goshua G, Bendapudi PK. Evidence-based minireview: should caplacizumab be used routinely in unsolicited patients with immune thrombotic thrombocytopenic purpura? *Hematol Am Soc Hematol Educ Program*. 2022;2022:491–494. doi:10.1182/hematology.2022000412
8. Volker LA, Balduin G, Kuhne L, et al. Caplacizumab is effective in treating immune-mediated thrombotic thrombocytopenic purpura in real-world scenarios and may lower mortality: an aggregative analysis. *Blood*. 2021;138(Suppl 1):1019. doi:10.1182/blood-2021-153211
9. Kuhne L, Kaufeld J, Volker LA, et al. Alternate-day dosing of caplacizumab for immune-mediated thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2022;20:951–960. doi:10.1111/jth.15637



Validation of the International Consensus Group Criteria for Slide Review Following Automated Complete Blood Count at Jimma Medical Center, Ethiopia

Girum Tesfaye Kiya, Aklilu Getachew Mamo, Sintayehu Asaye Biya, Dejene Gebre Gobena, Natal Demeke & Tilahun Yemane Shenkutie

To cite this article: Girum Tesfaye Kiya, Aklilu Getachew Mamo, Sintayehu Asaye Biya, Dejene Gebre Gobena, Natal Demeke & Tilahun Yemane Shenkutie (2023) Validation of the International Consensus Group Criteria for Slide Review Following Automated Complete Blood Count at Jimma Medical Center, Ethiopia, Journal of Blood Medicine, , 213-220, DOI: [10.2147/JBM.S402940](https://doi.org/10.2147/JBM.S402940)

To link to this article: <https://doi.org/10.2147/JBM.S402940>



© 2023 Kiya et al.



Published online: 24 Mar 2023.



Submit your article to this journal



Article views: 400



View related articles



View Crossmark data

Validation of the International Consensus Group Criteria for Slide Review Following Automated Complete Blood Count at Jimma Medical Center, Ethiopia

Girum Tesfaye Kiya ¹, Aklilu Getachew Mamo ¹, Sintayehu Asaye Biya ¹, Dejene Gebre Gobena¹, Natal Demeke², Tilahun Yemane Shenkutie¹

¹School of Medical Laboratory Science, Health Institute, Jimma University, Jimma, Ethiopia; ²Washington Medical Center, Addis Ababa, Ethiopia

Correspondence: Girum Tesfaye Kiya, P.O.Box: 378, Tel +251920274035, Email girumtesfaye12@gmail.com

Background: The international consensus group suggested criteria for action following automated complete blood count and white blood cell differential analysis. These criteria were set based on data from laboratories of developed countries. It is highly important to validate the criteria in developing countries where infectious diseases are still rampant that can affect blood cell count and morphology. Thus, this study aimed to validate the consensus group criteria for slide review at Jimma Medical Center, Ethiopia from November 1, 2020 to February 30, 2021.

Methods: The study comprised a total of 1685 patient samples from the daily laboratory workload of CBC analysis. The samples were collected in K2-EDTA tubes (Becton Dickinson) and analyzed using Coulter DxH 800 and Sysmex XT-1880 hematology analyzers. A slide review was done on two Wright-stained slides for each sample. All statistical analyses were performed using SPSS version 20 software.

Results: There were 39.8% positive findings, the majority of which were related to red blood cells. The false negative and false positive rates for Sysmex and Coulter analyzer were 2.4% vs 4.8%; and 4.6% vs 4.7%, respectively. The false negative rate was unacceptably higher when we used physicians' triggered slide review, which was 17.3% and 17.9% for Sysmex and Coulter analyzers, respectively.

Conclusion: Generally, the consensus group rules are suitable to use in our setting. However, we might still need to modify the rules, particularly to reduce the review rates. It is also necessary to confirm the rules with case mixes proportionally derived from the source population.

Keywords: validation, international consensus group criteria, slide review, complete blood count

Introduction

Automations in hematology laboratory changed the discipline dramatically following the introduction of a variety of analyzers that provide a reliable and accurate blood cell count, as a complete blood count (CBC).¹ CBC is a test that measures the number and physical characteristics of cells in the circulation including white blood cells (WBC), red blood cells (RBC), and platelets, to determine the general health status of the patient and screen, diagnose and monitor a variety of diseases and conditions that affect blood cells.²

Generally, there are nine components of CBC named as white WBC count, RBC count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hgb (MCH), mean corpuscular Hgb content (MCHC), platelet count, and red cell distribution width (RDW). Depending on the type of analyzer, some of these parameters are directly measured, such as WBC, RBC, Hgb, and MCV, while others are calculated such as Hct.³ With the advancement of automation, quantitative and qualitative information are becoming available in the form of flags,

histograms, or scattergrams. In addition to the traditional CBC parameters. Abnormalities in one or more components may indicate the presence of one or more conditions, which often leads to other tests to determine the cause of abnormalities, usually the peripheral blood film (PBF) examination.²

A PBF is a laboratory procedure that involves the cytology of peripheral blood cells smeared on a slide. The diagnostic importance of PBF is enormous and its relevance has not been lessened by advances in hematology automation.⁴ It is an inexpensive but powerful diagnostic tool and in some cases is sufficient to establish a diagnosis alone.⁵

However, some evidence recommended that there is a necessity of reducing PBF examination in light of time and monetary savings,⁶ and others suggested that the CBC and PBF are well correlated and hence the usage of automation is recommended to ease workload and time for patients.⁷

On the other hand, some evidence showed a significant discrepancy between automation and PBF on some parameters and suggested the interpretation of CBC results in light of the PBF examination.^{8,9} In addition to its importance in quality assurance to confirm findings of automation, PBF is found to be crucial to solely detect some important morphologic changes.¹⁰

In most resource-limited areas, PBF following CBC is performed without any objective criteria and is mainly triggered by physicians. In 2005, the international consensus group (ICG) led by Dr Berend Houwen suggested criteria for action following automated CBC and WBC differential analysis.¹¹ The group indicated the importance of these criteria for a wide range of hematology laboratories and suggested validation of the rules in individual laboratories before implementation as one size does not fit all due to various reasons like the practice of individual clinics, test volume, the instrument used, and others. The ICG criteria were established based on data from laboratories of developed countries including the US, Canada, Australia, and Europe. Hematological tests are often influenced by different variables including race, age, and gender; dietary habits, exposure to pathogens, ecological factors; condition of assay, variations in instrumentation techniques and laboratory personnel.¹² Thus, it is highly important to validate the criteria in developing countries where infectious diseases are still rampant that can affect blood cell count and morphology. Thus, the current study aimed to validate the consensus group criteria for slide review following automated CBC at JMC, Ethiopia.

Materials and Methods

Study Setting

The study was conducted at Jimma Medical Center Hematology laboratory. The Medical Center is one of the largest health facilities in the country providing service to 20 million people with 1600 staff members and 800 beds. The average daily CBC analysis is about 200. The study was conducted from November 1, 2020 to February 30, 2021 comprising a total of 1685 blood samples from the daily laboratory workload of CBC analysis. To validate the ICG criteria, the minimal sample size suggested by the international society for laboratory hematology (ISLH) was 1000 [11]. An average of 20 samples were randomly selected from the daily workload. The samples were collected in K2-EDTA tubes (Becton Dickinson) and analyzed using Coulter DxH 800 and Sysmex XT-1800 hematology analyzers. Among the total samples, 792 were analyzed by Sysmex XT-1800 while 893 were analyzed by Coulter DxH 800 hematology analyzers.

Study Design

A cross-sectional study design was employed to validate the ICG criteria and to compare the criteria with the existing practice.

Peripheral Blood Film Review

A manual slide review was done on two Wright-stained slides for each sample by two experienced technicians, independently and blinded to the automation results. Positive results were defined based on the ISLH recommendation stated as follows.¹¹ The detailed version of the recommendation with grading system is attached in the [Supplementary Data](#) section ([Supplementary Data 1](#)).

Morphology

- (a) RBC morphology at 2+/moderate or greater. The only exception is malaria, where any finding will be considered a positive finding.
- (b) PLT morphology (giant platelets) at 2+/moderate or greater.
- (c) Platelet clumps at > rare/occasional.
- (d) Dohle bodies at 2+/moderate or greater.
- (e) Toxic granulation at 2+/moderate or greater.
- (f) Vacuoles at 2+/moderate or greater.

Abnormal Cell Types

- (a) Blast $\geq 1\%$
- (b) Metamyelocyte $> 2\%$
- (c) Myelocyte/promyelocyte $\geq 1\%$
- (d) Atypical lymphocyte $> 5\%$
- (e) NRBC (Nucleated RBC) $\geq 1\%$
- (f) Plasma cells ≥ 1

Quality Assurance

The analysis of all samples was according to the standard operating procedure and manufacturer's instructions. Internal quality control (IQC) of the analyzers was regularly applied every three month and an in-house developed patient sample was used as IQC every morning once per day. Moreover, three times a year, the JMC laboratory participates in an external quality assessment (EQA) run by one world accuracy of Canada, involving complete blood count and peripheral blood film review.

Statistical Analysis

All statistical analyses were performed using SPSS version 20 software. The automated CBC results were sorted into four categories as a truth table based on the correlation between the positive/negative status defined by the ICG rules ([Supplementary Data 2](#)) versus the "truth" defined by the slide review. If a rule was triggered, and the smear contained a positive finding, the sample was graded as true positive (TP). If a rule was triggered, but the smear did not contain any positive findings, the sample was graded as false positive (FP). A true negative (TN) grade was given when there was no triggered rule, and the smear did not contain any positive findings. A false negative (FN) grade was given when there was no triggered rule, but the smear contained a positive finding.¹¹

Sensitivity, specificity, accuracy rate, error rate, review rate, positive predictive values, and negative predictive values were calculated based on the following calculation:

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$$

$$\text{Specificity} = \text{TN} / (\text{FP} + \text{TN})$$

$$\text{Accuracy rate} = (\text{TP} + \text{TN}) / \text{Total}$$

$$\text{Error rate} = (\text{FP} + \text{FN}) / \text{Total}$$

$$\text{Review Rate} = (\text{TP} + \text{FP}) / \text{Total}$$

$$\text{Predictive Value of a Positive Result} = \text{TP} / (\text{TP} + \text{FP})$$

$$\text{Predictive Value of a Negative Result} = \text{TN} / (\text{FN} + \text{TN})$$

Ethical Considerations

Ethical clearance was obtained from Jimma University, Institute of Health Ethical Review Committee (Ref. No. IRB000342/2012). The principles of the Declaration of Helsinki have been followed in conducting the study. Any information concerning the participants was kept confidential and the Authors had no access to information that could identify individual participants during or after data collection. The patient result of the slide review was communicated to the treating physician.

Results

The samples were collected from more than 13 wards including medical, outpatient, emergency, pediatrics, maternity, and oncology. About a fourth of all samples (27%) came from oncology department, whilst samples from ICU and ART clinic were the least frequent. From the total samples, 45% were from males and 55% were from females. The age of the patients ranges from 1 to 90 years.

Analysis of Smear Review Findings

Out of the total 1685 samples, there were 671 (39.8%) positive findings according to the definition of ISLH; 283 (42%) were positive samples from Coulter analyzer, whereas 388 (58%) were positive samples from Sysmex analyzer. The majority of the abnormalities were related to RBC, followed by WBC and platelet abnormalities. Anisocytosis was the most common finding while giant platelet is the least common.

Analysis of the ICG Criteria

The truth table showed that FN and FP results were 2.4% and 4.8%, respectively, for Sysmex analyzer. For Coulter analyzer, the FN and FP rates were 4.6% and 4.7%, respectively. The FN rate was within the acceptable range for both analyzers which is less than 5%. The review rate was also found to be 51% and 31.8% for the two analyzers, respectively. The diagnostic performance of both analyzers based on the ICG criteria and slide review positivity was found to be good with sensitivity and specificity of 95.1% and 90.6% on Sysmex and 85.5% and 93.1% on Coulter analyzer (Table 1).

False Negative

The total number of FN occurrences on Sysmex and Coulter analyzer were 24 and 57 and the total number of FN cases were 19 and 41, respectively. The majority of the occurrences on samples from coulter analyzer and Sysmex analyzer were related to RBC abnormalities, particularly, anisocytosis followed by findings of macrocytes. Moreover, white blood cell abnormalities, such as toxic granulation and atypical lymphocyte contributed to the FN rate (Figure 1).

Table 1 Truth Table Based on the International Consensus Group Criteria

Status	Instruments	
	Sysmex	Coulter
True negative	365 (46.1%)	564 (63.2%)
True positive	369 (46.6%)	242 (27.1%)
False negative	19 (2.4%)	41 (4.6%)
False positive	38 (4.8%)	42 (4.7%)
Review rate	407 (51%)	284 (31.8%)
Sensitivity	95.1%	85.5%
Specificity	90.6%	93.1%
Positive predictive value	90.6%	85.2%
Negative predictive value	95.1%	93.2%
Accuracy rate	92.7%	90.3%
Error rate	7.2%	9.3%

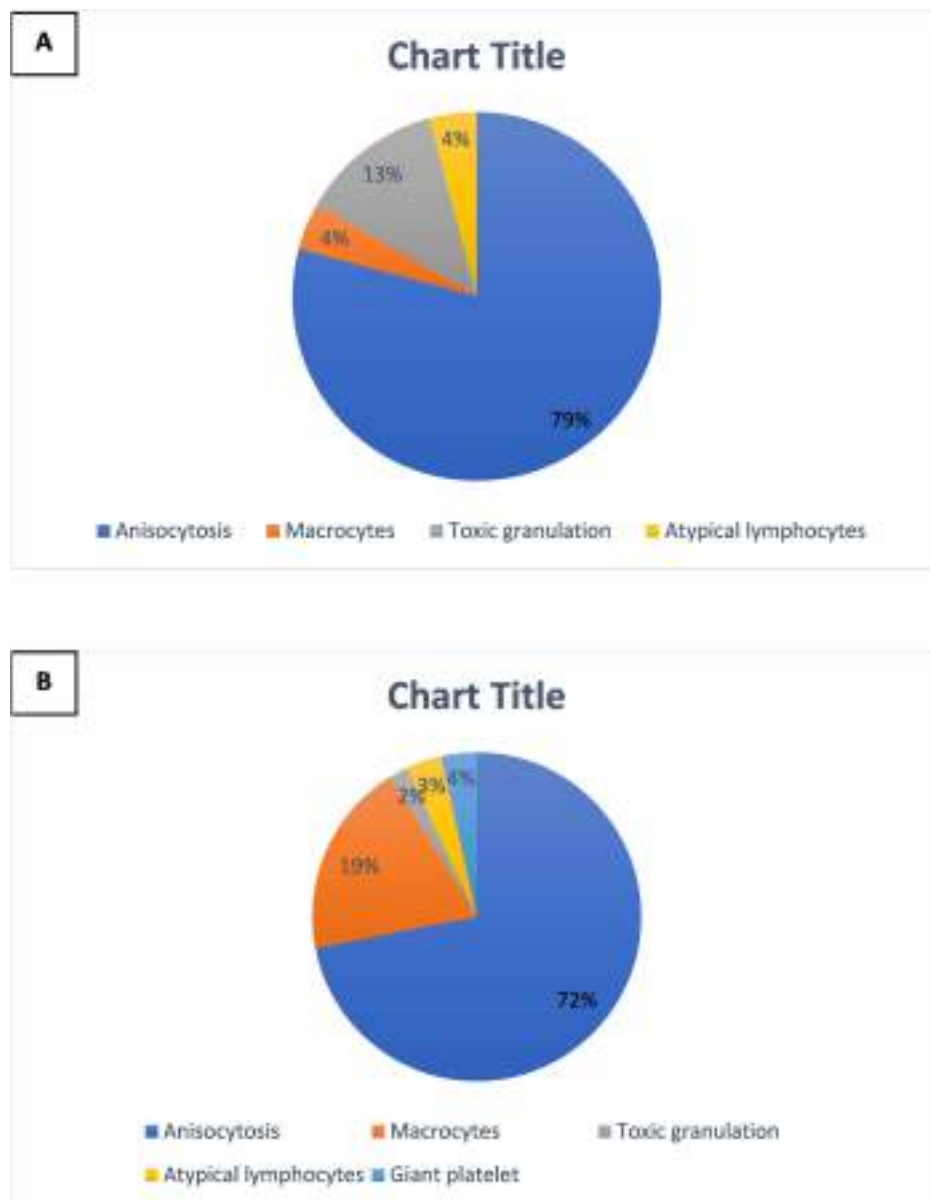


Figure 1 False negative occurrences on Sysmex (A) and Coulter analyzer (B).

False Positive and True Positive

The most frequently triggered rule on Sysmex analyzer was rule number 5 followed by rule no. 7, 9, and 10. Rule no. 7 was the most frequently triggered rule on Coulter analyzer followed by rule no. 9, 10, and 5. Generally, the TP rate was higher on Sysmex analyzer than Coulter analyzer. In total, 18 cases triggered rule no. 5 (WBC count) on Sysmex analyzer, the majority (17/18) of which appears to be because of the neutrophil count (rule no. 17). The same is true on Coulter analyzer where 10 cases triggered rule no. 5 and 19 cases triggered rule no. 17. The second most common triggered rule among the FP cases was rule no. 7 (platelet count) which was 17 and 16 cases on Sysmex and Coulter analyzer, respectively (Table 2).

Comparison Between the Consensus Group Criteria and Physicians' Triggered Slide Review

From the total samples included in the study, 616 (36.5%) slide reviews were triggered by physicians. The truth table based on physicians' triggered slide reviews showed different results on both analyzers. The FN rate was unacceptably

Table 2 True Positive and False Positive

Rule no.	Parameters	Rules	Sysmex		Coulter	
			FP Rate, n (%)	TP Rate, n (%)	FP Rate, n (%)	TP Rate, n (%)
5	WBC	<4x10 ⁹ /L or >30 x10 ⁹ /L	18 (47.4)	141 (38.4)	10 (23.8)	80 (33)
7	PLT	<100x10 ⁶ /L or >1000 x10 ⁶ /L	17	140	16	105
9	HGB	<7g/dl or >2g/dl from upper reference range for age and sex	9	135	3	92
10	MCV	<75fl or >105fl	6	130	8	88
17	Neutrophil	<2x10 ⁹ /L or >20x10 ⁹ /L	17	121	19	77
15	RDW	>22	8	87	3	65

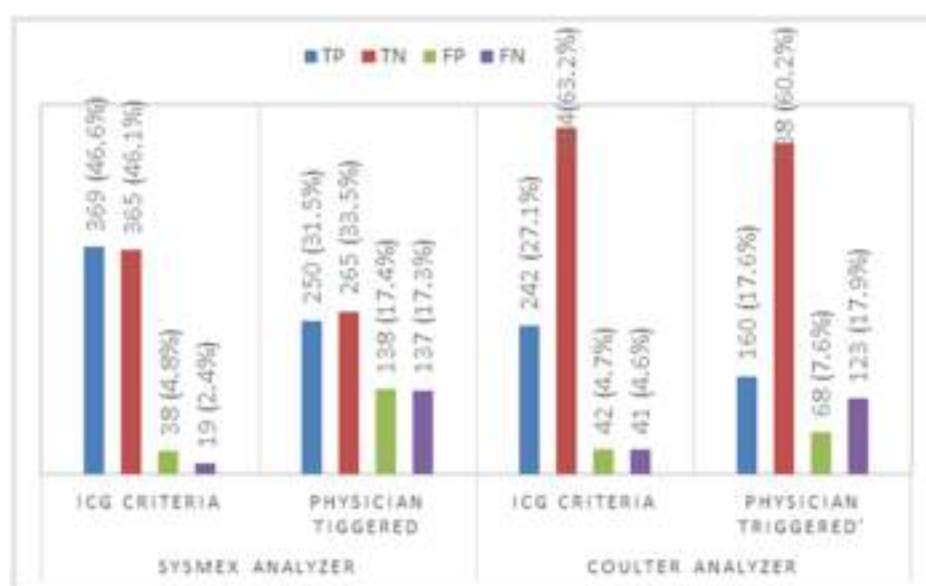
Abbreviations: WBC, white blood cell; PLT, platelet; HGB, hemoglobin; MCV, mean cell volume; RDW, red cell distribution width; FP, false positive; TP, true positive.

higher on both analyzers as compared to the ICG criteria (Figure 2). The review rate was also found to be 49% and 25.5% on Sysmex and Coulter analyzers, respectively.

Discussion

A consensus group established by the ISLH suggested indications for slide review and/or manual WBC differential count following automated CBC.¹¹ The group was comprised of 20 experts from multiple developed countries who use multiple instruments. Criteria at participants' laboratories were discussed and finally combined into 41 rules. These rules have to be tested in different laboratories in the world before use. The present study involved 1685 samples analyzed by two automated instruments at JMC in Ethiopia to test if the recommended criteria are well fitting to laboratories of developing countries.

In this study, evaluation of the ICG review criteria yielded an FN rate of 2.4% and 4.6% for Sysmex and Coulter analyzers, respectively. These results were within the acceptable range according to the ICG recommendation of a maximum acceptable FN rate of less than 5%.¹¹ Though anisocytosis is the most common finding that caused FN results, adjustment of rule #15 (RDW rule) may not be necessary as the rate is within the acceptable range. The FP rate on both analyzers was also acceptable when compared to the rate that was found by the ICG, which is 18.6%. Rule #5 and # 17 were the most frequently triggered rules that caused FP results. Increased WBC count (>30 x10⁹/L) rather than

**Figure 2** Comparison between the consensus group criteria and physicians' triggered slide review.

decreased WBC ($<4 \times 10^9/L$), particularly the neutrophil count ($>20 \times 10^9/L$) was the frequent reason as expected in the areas where bacterial infection is common.¹³

The College of American Pathologists (CAP) had a Q-probe study of 95,151 CBC determinations performed in 263 institutions and the median PBF review rate was 26.7%, which was suggested as a normative review rate.¹⁴ The present study showed that the review rate for Sysmex and Coulter analyzers were 51% and 31.8%, respectively. The review rate of samples from Sysmex analyzer appears to be higher as compared to the review rate of the Q-probe study by CAP. However, a lower review rate, though desirable, might say nothing about quality. In our case, the TP rate was higher in samples from Sysmex analyzer as compared to the samples analyzed by Coulter. This might be due to the patient mix analyzed by this analyzer, the majority of which are from oncology and emergency department, as compared to the samples analyzed by Coulter machine.

The comparison of our finding with previously published studies showed that the FN rate is higher than the previous findings, in which the rate range from 1.6 to 2.2,^{15,16} but lower than studies that reported FN rate ranging from 7.37 to 9.25.^{17,19} The FP rate in this study is much better than previous studies, which reported FP rates ranging from 7.2% to 34%.^{15,16,18,19} Moreover, the review rate in the present study was found to be comparable with some previous studies, which reported a review rate ranging from 29% to 51%.^{15,16,19,20}

This study has also compared the ICG criteria and physicians' triggered slide reviews. The physicians' triggered slide review practice appears to be arbitrary in the sense that no specific rule is set following automated CBC to indicate slide review. The truth table showed that the FN rate was unacceptably higher among slide reviews triggered by physicians. This indicates that many positive findings are remaining unnoticed due to a lack of objective rules, which in turn might affect patients' safety.

Conclusion

Generally, the ICG rules set by ISLH are suitable to use in our setting. However, we might still need to modify the rules, particularly to reduce the review rates in light of a shortage of skilled technicians to review large amounts of samples. On the other hand, though the review rate was desirably lower for samples from the Sysmex analyzer, continuing with physicians' triggered slide review approach might result in increased unnoticed positive findings. It is also necessary to confirm the rules with case mixes proportionally derived from the source population.

Acknowledgments

The authors would like to thank Jimma University's research and community service office for supporting this study. We sincerely thank the staff of Jimma University Medical Center laboratory unit for their unreserved support during the study.

Funding

This study was supported by the Jimma University research and community service office for data collection, analysis, and resources; no authors received special or specific funding for this study.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Kathy WJ. *Evaluation of Cell Morphology and Introduction to Platelet and White Blood Cell Morphology*. Philadelphia PA: FA. Davis. Company; 2010.
2. Complete blood count. Lab tests online; 2022. Available from: <https://labtestsonline.org.uk/tests/full-blood-count-fbc>. Accessed March 21, 2023.
3. Betty C. *Hematology in Practice*. F.A Davis Company; 2010.
4. Adewoyin AS, Nwogoh B. Peripheral blood film - a review. *Ann Ib Postgrad Med*. 2014;12(2):71–79.
5. Bain BJ. Diagnosis from the blood smear. *N Engl J Med*. 2005;353(5):498. doi:10.1056/NEJMr043442
6. Joubert J, Weyers R, Raubenheimer J. Reducing unnecessary blood smear examinations: can Sysmex blood cell analysers help. *Med Technol SA*. 2014;28:6–12.

7. Ike SO, Nubila T, Ukaejiofo EO, Nubila IN, Shu EN, Ezema I. Comparison of haematological parameters determined by the Sysmex KX - 2IN automated haematology analyzer and the manual counts. *BMC Clin Pathol*. 2010;10(1):1–5. doi:10.1186/1472-6890-10-3
8. Poonam R. Automated red blood cell analysis compared with routine RBC morphology by smear review. *Blood*. 2011;56:34–39.
9. Aseem Jain A, Bhake MT. Co-relative study on peripheral blood smears in anemia with automated cell counter generated red cell parameters. *Semant Sch*. 2018;2018:1.
10. Rosenthal DS. Evaluation of the peripheral blood smear – upToDate; 2022. Available from: https://www.uptodate.com/contents/evaluation-of-The-peripheral-blood-smear?search=peripheralbloodfilm&usage_type=default&source=search_result&selectedTitle=1~150&display_rank=1. Accessed March 21, 2023.
11. Barnes PW, McFadden SL, Machin SJ, Simson E. The international consensus group for hematology review: suggested criteria for action following automated CBC and WBC differential analysis. *Lab Hematol*. 2005;11(2):83–90. doi:10.1532/LH96.05019
12. Bimerew LG, Demie T, Eskinder K, et al. Reference intervals for hematology test parameters from apparently healthy individuals in southwest Ethiopia. *SAGE Open Med*. 2018;6:205031211880762. doi:10.1177/2050312118807626
13. Honda T, Uehara T, Matsumoto G, Arai S, Sugano M. Neutrophil left shift and white blood cell count as markers of bacterial infection. *Clin Chim Acta*. 2016;457:46–53. doi:10.1016/j.cca.2016.03.017
14. Novis DA, Walsh M, Wilkinson D, St. Louis M, Ben-Ezra J. Laboratory productivity and the rate of manual peripheral blood smear review: a college of American pathologists Q-probes study of 95,141 complete blood count determinations performed in 263 institutions. *Arch Pathol Lab Med*. 2006;130(5):596–601. doi:10.5858/2006-130-596-LPATRO
15. Pratumnvinit B, Wongkrajang P, Reesukumal K, Klinbua C, Niamjoy P. Validation and optimization of criteria for manual smear review following automated blood cell analysis in a large university hospital. *Arch Pathol Lab Med*. 2013;137(3):408–414. doi:10.5858/arpa.2011-0535-OA
16. Pipitone S, Germagnoli L, Da Rin G, et al. Comparing the performance of three panels rules of blood smear review criteria on an Italian multicenter evaluation. *Int J Lab Hematol*. 2017;39(6):645–652. doi:10.1111/ijlh.12720
17. Eldanasoury AS, Boshnak NH, Abd RE, Monem E. Validation of criteria for smear review following automated blood cell analysis in Ain Shams university laboratory. *Int J Sci Res*. 2013;5:2319–7064.
18. Buoro S, Mecca T, Seghezzi M, et al. Validation rules for blood smear revision after automated hematological testing using Mindray CAL-8000. *J Clin Lab Anal*. 2017;31(4):e22067. doi:10.1002/jcla.22067
19. Comar SR, Malvezzi M, Pasquini R. Are the review criteria for automated complete blood counts of the international society of laboratory hematology suitable for all hematology laboratories? *Rev Bras Hematol Hemoter*. 2014;36(3):219. doi:10.1016/j.bjhh.2014.03.011
20. Wang X, Wang X, Ge P, et al. Establishment of improved review criteria for hematology analyzers in cancer hospitals. *J Clin Lab Anal*. 2021;35(2):e23638.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Critical Assessment of Transfusional Practices in the Obstetrics and Gynecology Department At the University Hospital of Kinshasa

Yvon Wangi Ngoy, Mireille Solange Nganga Nkanga, Guy Ndelembo Baina, Audrey Katende Katende, Jacques Bikaula Ngwidiwo, Lethy Mpudi Massamba, Fanny Walo Diessa, Bienvenu Kuyangisa Boloko, Héritier Mawalala Malengele & Benjamin Longo-Mbenza

To cite this article: Yvon Wangi Ngoy, Mireille Solange Nganga Nkanga, Guy Ndelembo Baina, Audrey Katende Katende, Jacques Bikaula Ngwidiwo, Lethy Mpudi Massamba, Fanny Walo Diessa, Bienvenu Kuyangisa Boloko, Héritier Mawalala Malengele & Benjamin Longo-Mbenza (2023) Critical Assessment of Transfusional Practices in the Obstetrics and Gynecology Department At the University Hospital of Kinshasa, Journal of Blood Medicine, , 221-231, DOI: [10.2147/JBM.S381016](https://doi.org/10.2147/JBM.S381016)

To link to this article: <https://doi.org/10.2147/JBM.S381016>



© 2023 Wangi Ngoy et al.



Published online: 27 Mar 2023.



Submit your article to this journal



Article views: 305



View related articles



View Crossmark data

Critical Assessment of Transfusional Practices in the Obstetrics and Gynecology Department At the University Hospital of Kinshasa

Yvon Wangi Ngoy¹⁻³, Mireille Solange Nganga Nkanga^{1,2}, Guy Ndelembo Baina¹, Audrey Katende Katende³, Jacques Bikaula Ngwidiwo¹, Lethy Mpudi Massamba¹, Fanny Walo Diessa¹, Bienvenu Kuyangisa Boloko¹, Héritier Mawalala Malengele^{2,4}, Benjamin Longo-Mbenza^{2,4,5}

¹Department of Medical Biology, University Hospital of Kinshasa, University of Kinshasa, Kinshasa, Democratic Republic of Congo; ²Lomo University of Research, Kinshasa, Democratic Republic of Congo; ³Department of Medical Biology, Institute for Health Science Research, Kinshasa, Democratic Republic of Congo; ⁴Department of Internal Medicine, University Hospital of Kinshasa, University of Kinshasa, Kinshasa, Democratic Republic of Congo; ⁵Department of Health, Walter Sisulu University, Mthatha, South Africa

Correspondence: Benjamin Longo-Mbenza, Faculty of Health Science, Private Bay XI, Mthatha, Eastern Cape, 5117, South Africa, Tel +243 814396257, Email longombenza@gmail.com

Introduction: Gynecology and obstetrics are among the specialties where blood transfusion is most practiced. This situation requires the use of good transfusion practices. The objective of this study was to assess the quality of transfusion practice in the Gynecology and Obstetrics Department of the University Hospital of Kinshasa (UHK).

Methods: This is a prospective, evaluative and descriptive study performed at the Department of Gyneco-Obstetrics of the University Hospital of Kinshasa from February 25 to June 25, 2020; which dealt with patients who received at least one blood transfusion.

Results: About 498 patients, 54 patients were transfused and their average age was 36.4 years, with the extremes of 14 and 60 years, the transfusion rate was 10.8%. Most of patients (n=36: 2/3) were transfused during weekend days, sachets were used as delivery materials of blood product in 57.4% of cases (n = 31). Among the prescribers of blood products, 70.4% were nurses. All transfusions were carried out in type-specific and cross-matched Rh. All the transfused patients were not aware of the disadvantages of transfusion. Compatibility tests at the patient's bedside were not performed in 61.1% of cases and the concordance control between the patient and the blood component to be transfused was not performed in 70.4% of cases. Close monitoring of blood transfusion in the first ten minutes was not performed in 59.3% of cases.

Conclusion: Transfusion encounters real practical problems in the gyneco-obstetrical environment of countries with limited resources. However, an assessment and multidisciplinary collaboration would be necessary to improve transfusion practice in medical field.

Keywords: assessment, transfusion practices, gyneco-obstetrics, University Hospital of Kinshasa

Introduction

The practice of transfusion is common in Gynecological and Obstetrical specialties, because of a high frequency of complications such as: disorders of parturition, incomplete abortions, cases of placenta accreta and rupture of the uterus, caesarean section, close-term anemia, ruptured ectopic pregnancy, evacuation of hydatid moles and cancer (uterus, breast, ovary).¹⁻³ As a result, blood transfusion remains a daily concern in these two specialties.⁴ In addition, the transfusion and post-transfusion risk occurred requires the use of good transfusion practices.⁵ Unfortunately, the act of transfusion is often marred by non-conformities confirmed by several studies carried out in SSA. These studies revealed non-conformities in relation to bedside compatibility tests (72.6%),^{2,6} the concordance control of the blood group mentioned on the patient's file with the one mentioned on the blood product (15.5%)^{1,2} and close monitoring of blood transfusion during the first ten minutes (88.3%).²

Then, to secure the act of transfusion in countries with limited resources, the World Health Organization (WHO) recommended in 2016 to strengthen the management of the quality system throughout the transfusion chain and to carry out training programs and performing internal and external assessment of quality systems.^{7,8} To carry out these assessments, collaboration between clinicians and biologists is necessary, but also strategies to reduce the inappropriate use of blood and blood components in all specialties.⁸ These strategies include guidelines, monitoring of transfusion practice and self-assessment by clinicians.³ The objective of this study was to assess the quality of transfusion practices in the Gynecology and Obstetrics Departments of the UHK.

Methods

This is a prospective, evaluative and descriptive study performed over a period of 4 months, from February 25 to June 25, 2020 in the Gyneco-Obstetrics Department of the University Hospital of Kinshasa. The study population is composed of patients admitted to the Gyneco-Obstetrics Department of the UHK and having been transfused at least once during the study period.

Sampling

An exhaustive and reasoned sampling of patients transfused during the study period was carried out (n=54).

Variables of Interest

The different variables of interest were obtained by direct observation of transfusion performed by the health agents who practiced transfusion on the one hand, and by questioning the patients or their members on the other hand.

The parameters of interest were as follows: age, profession, marital status, days of transfusion, materials for transporting blood components, information on the advantages and disadvantages of blood transfusion before transfusion act, the practitioners of the blood transfusion, the concordance control between the patient and the blood component to be transfused, the type of transfusion, the compatibility test at the patient's bedside, monitoring of the blood transfusion in the first 10 minutes and the acute transfusion reactions.

Operational Definitions

Critical assessment was defined as a systematic and critical analysis of the quality of medical care by comparing procedures or services with the standard references, in order to make corrections.

Appropriate blood component was a blood component transfused solely to treat a condition leading to significant morbidity and mortality that cannot be prevented or effectively managed by other means.⁵

Statistical Analyzes

Categorical data were presented by their numbers (n) and their percentages (proportions). Associations between categorical variables were sought by Pearson's Chi-square test.

The normality of the quantitative variables was assessed by the Kolmogorov-Smirnov test. Quantitative variables were characterized by the mean $\pm$ standard deviation ($X \pm SD$) or the median with its interquartile (Med (EIQ)). The average of the ages with normal distribution was compared by Student's *t*-test between two groups but also with the analysis of variances (ANOVA) between ≥ 3 groups.

In univariate (bivariate) analysis, the association between two continuous or quantitative variables was determined by Pearson's *r* coefficient in case of normal distribution but also by Spearman's ρ coefficient in case of abnormal distribution.

The statistical significance threshold was set at $p < 0.05$.

All statistical analyzes were performed with SPSS 23 software.

Results

Transfusion Rate

Upon a total of 498 patients admitted to the Gynecology and Obstetrics Departments, 54 patients received at least one blood transfusion, about 10.8%.

Age of Patients

Table 1 sums up the central tendency, the dispersion, the quartile and the retrospective distribution ages of the study population. In fact, the range of age extremes (minimum = 14 years and maximum = 60 years) was spread out and categorized according to the chronobiology of the following population: adolescent-young adult with age <34 years versus advancing into adulthood with age ≥ 34 years. Most of our patients (51.9%) were between 14 and 33 years old (Table 2).

Table 1 Distribution of Data Related to the Compliance Parameters of the Transfusion Rules

Parameters		Frequency	Percentages
Disadvantages of the BT	No	54	100.0%
	Yes	0	0.0%
Advantages of BT	No	16	29.6%
	Yes	38	70.4%
Concordance control patient/ LBC to be transfused	No	38	70.4%
	Yes	16	29.6%
Compatibility test at Patient's bedside	No	33	61.1%
	Yes	21	38.9%
Monitoring BT during the first 10 minutes	No	32	59.3%
	Yes	22	40.7%
Adverse effects	No	13	24.1%
	Yes	41	75.9%
Death	No	47	87.0%
	Yes	7	13.0%

Table 2 Distribution of the Patients Related to the Age of the Study Population

Statistics		AGE (Years)
N	Valid	54
	Missing	0
Average		36.4
Standard error of the average		1.8

(Continued)

Table 2 (Continued).

Statistics		AGE (Years)
Median		33.5
Mode		60.0
Standard deviation		13.0
Skewness		0.5
Skewness standard error		0.3
Kurtosis		-0.9
Kurtosis standard error		0.6
Minimum		14
Maximum		60
Percentiles	25	25.0
	50	33.5
	75	46.0

Patient Profession

Among all patients 32, 8.1, 2.1, 2.3, 1.2 and 2 had reported the professions of housewife, student/pupil, demographer, policewoman, doctor, seamstress, trader, magistrate and nurse: most of the patients (close to 60% and 2/3) being less educated housewives than the other professions.

Transfusion Days

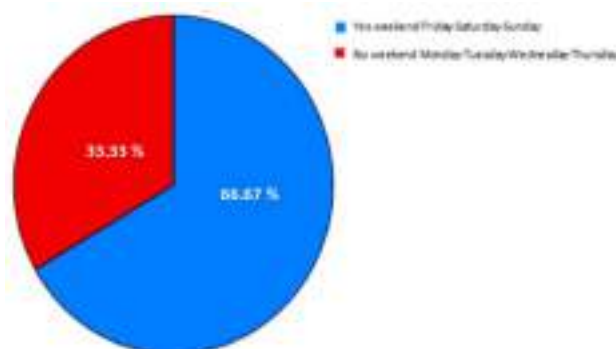
The days of blood transfusions characterized the frequencies of the study population: most of patients (n=36: 2/3) were transfused during weekend days (Friday-Saturday-Sunday) against 18 patients transfused during non-weekend days (Figure 1).

Indicator for the Critical Assessment of Transfusion Practices

Materials for Transporting Blood Products

Materials for transporting blood products were in the form of a sachet in 57.4% of cases (n = 31), in the form of a cardboard box in 33.3% of cases (n = 18), by the hands of family members in 9.3% of cases (n=5) and with a thermo stable cooler in 0.0% of cases (n=0) (Figure 2).

Haemorrhage was the main clinical indication for blood transfusions in the present study. This was dominated by postpartum haemorrhage (42.6%) of cases (n=23), followed by post oncological surgery haemorrhage (37.0%) of cases (n=20), and pre-intrapartum haemorrhage (20.4%) of cases (n=11).

**Figure 1** Distribution of data related to the days of the week that transfusion occurred.

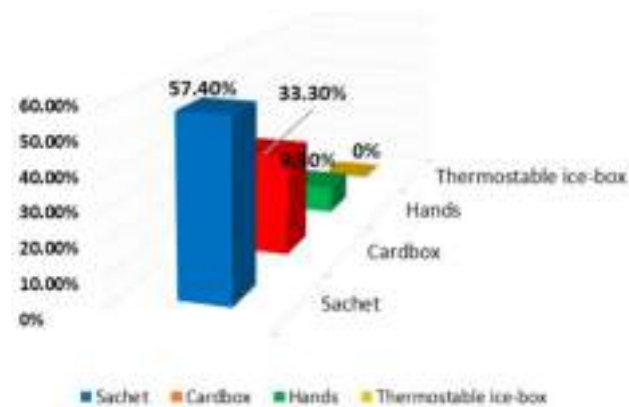


Figure 2 Distribution of data related to the transporting materials of the blood components.

Practitioners of the Blood Transfusion

Among practitioners of Blood Transfusion, 70.4% of nurses (n=38) were comparable to 29.6% of resuscitator physicians (n=16) (Figure 3).

Type of Transfusion

Homologous transfusion (type-specific and cross-matched Rh) as type of transfusion was performed in 100.0% of patients (n=54) (Figure 4).

Observance of Haemovigilance Rules

The parameters of compliance with the rules of blood transfusion were summarized in Table 1. This consisted of information on the advantages and disadvantages of blood transfusion to patients before the transfusion procedure, the control of concordance between the patient and the blood component unit to be transfused, compatibility testing at the patient's bedside, monitoring of blood transfusion in the first 10 minutes and adverse effects occurring during transfusions.

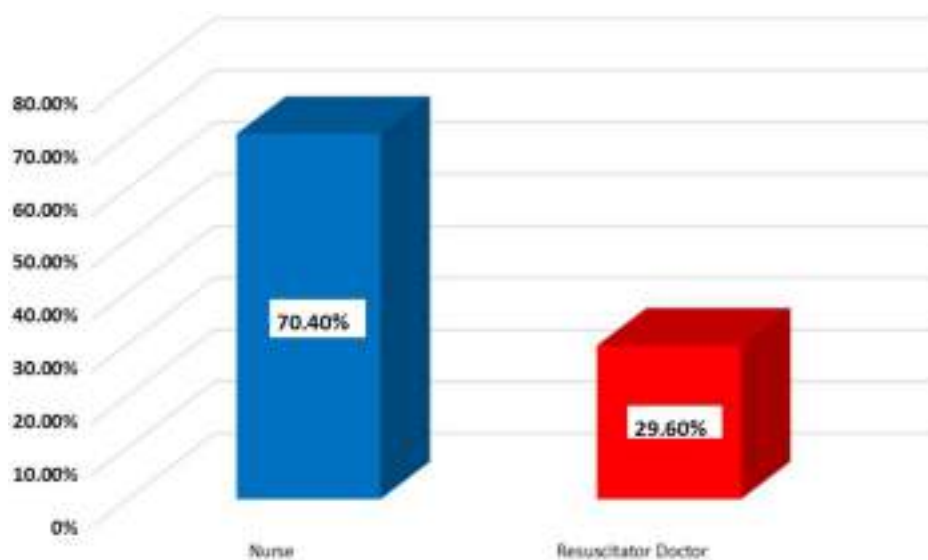


Figure 3 Distribution of data related to practitioners of blood transfusion.

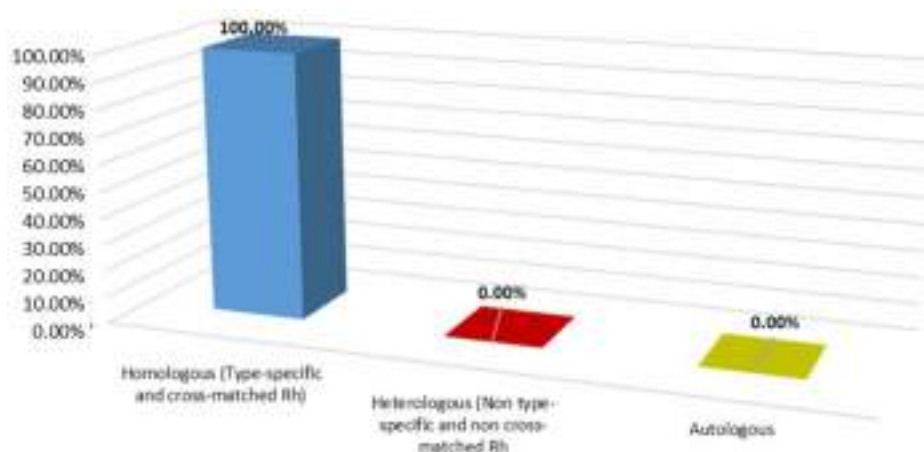


Figure 4 Distribution of data related to the type of transfusion. Homologue (iso groupe et iso rhesus): Homologous (Type-specific and cross-matched Rh). Hétérologue (Hétéro groupe et hétéro rhesus): Heterologous (Non type-specific and non cross-matched Rh. Autologue: autologous.

Univariate Analysis for the Adverse Effects as Predictor Occurring During Transfusions

Age and Acute Transfusion Reactions

All patients with the age advancement presented an acute transfusion reaction (average age ≥ 34 years, 100% $n=26$). Patients with an acute transfusion reaction were statistically older (median age = 41.2 ± 11.2 years; $P < 0.0001$) than their partners without a minor or major acute transfusion reaction (average age = 21.6 ± 3.6 years) (Figure 5).

Transfusion Days and Acute Transfusion Reaction

There was a highly significant association ($P < 0.001$) between the rates of acute transfusion reaction and the days when blood transfusions were performed during the week in the form of an exponential curve. Then, the acute transfusion reaction rate was

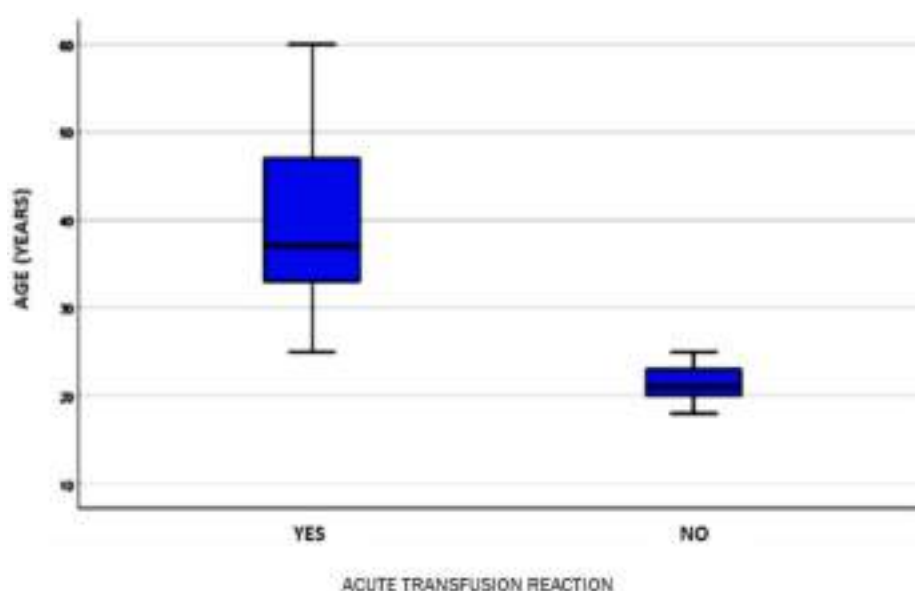


Figure 5 Distribution of data related to the interaction between age and acute transfusion reaction.

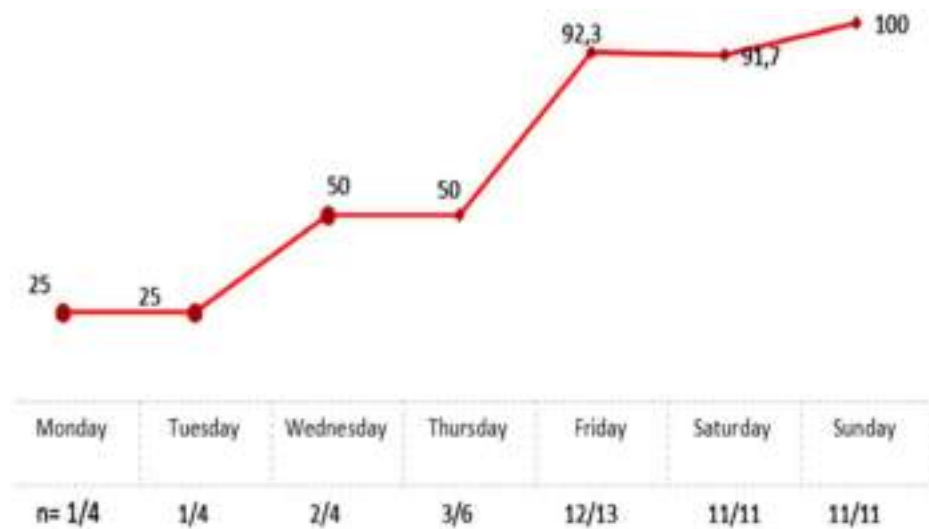


Figure 6 Distribution of data related to the adverse effects occurred during transfusions between the days of the week.

more frequent during weekend days (Friday-Saturday-Sunday) (94.4% $n=34/36$ RR 2.4 95% CI 1.4–4.4; $P < 0.001$) than during non-weekend working days (38.9% $n=4/18$) (Figure 6). The most prevalent reaction was hyperthermia reaction (Table 3).

Table 3 Days, Months and Reactions Post Transfusion

Parameters				Frequency	Percentage
February	Days	Monday	Thrill	1	2.4
		Thursday	Hyperthermia	1	2.4
		Saturday	Pruritus	2	4.9
			Hyperthermia	1	2.4
March	Days	Friday	Hyperthermia	4	9.8
			Pruritus	1	2.4
		Sunday	Thrill	1	2.4
			Pruritus	2	4.9
April		0	0	0	0.0
May	Days	Tuesday	Hyperthermia	1	2.4
		Wednesday	Hyperthermia	1	2.4
		Friday	Pruritus	1	2.4
			Hyperthermia	2	4.9
		Saturday	Thrill	2	4.9
			Icterus	1	2.4
		Sunday	Hyperthermia	3	7.3
			Pruritus	1	2.4
June	Days	Wednesday	Thrill	1	2.4
		Thursday	Thrill	1	2.4
			Urticaria	1	2.4
		Friday	Thrill	2	4.9
			Icterus	1	2.4
			Pruritus	1	2.4
		Saturday	Hyperthermia	2	4.9
			Pruritus	1	2.4
			Urticaria	2	4.9
		Sunday	Hyperthermia	1	2.4
			Pruritus	1	2.4
			Thrill	1	2.4
			Icterus	1	2.4

Discussion

This is the first study that assessed practices of transfusion in a gynecology-obstetrics environment at the UHK.

Transfusion Rate

In the current study, transfusion was performed in 10.8% of patients admitted during the study period. This rate is more or less comparable to those found by Chowdhury⁹ in 2016 in India and by Zamané² in Burkina Faso in 2014, which were respectively 9.2% and 11.1%. But it is higher than the rate indicated by Yung-Taek Ouh (1.9%)¹⁰ in Korea in 2014 and by Matthew Cauldwell (0.3%)¹¹ in the United Kingdom in 2017 in patients transfused in Gynecology-Obstetrics environment.

The relatively high rate observed in this study testifies an increased transfusion activity in the Gynecology-Obstetrics Department of the UHK. Indeed, the low socio-economic standard of patients (74.1%) constitutes an impasse to the early diagnosis and management of pathologies with a high risk of bleeding. In addition, pathophysiological aspects are generally not taken into account when prescribing BT: transfusion decisions are often made by non-trained practitioners and are based on limited and often low quality evidence. In addition, there is no evidence practice about any level of anemia.¹² It is also important not to consider blood transfusion (transfusion of allogeneic red blood cells, platelet concentrates and plasma units) especially for patients with cancer, as a standard therapeutic arsenal used in the context of surgical and non-surgical treatment.¹³

This rate is related to the organization of the night work services in GO/UHK during the weekend. At weekend, the number of doctors is less, supervisors on call are not on site most of the time, and consequently decisions are made without supervisors' opinion. In addition, patients are admitted urgently at weekend (81.5%) in an unstable hemodynamic state leading to an urgent transfusion. All these facts justify the increased rate of BT during the three days of the weekend at the GO/UHK.

In intermediate- and high-resource countries, some reports indicate improvements in transfusion practices (mainly with respect to the hemoglobin thresholds at transfused patients).^{3,5,14} These improvements are related to: the adoption of the restrictive strategy rather than the liberal strategy;¹² the personalization of the blood management (PBM); the mobilization of the means for training, the organization of mass voluntary blood donation campaigns and the organization of quality assessment.¹⁵

Age of Patients

The average age of our patients was around 33.5 years, this result is comparable to that reported in other studies performed in some African countries^{1,4,6,11,16} but further low than that of patients transfused in the departments of Gynecology and Obstetrics of developed countries.^{10,17}

Transport Equipment

The LBC transport equipment in the current study did not meet the recommendations of various standards in relation to the transport of LBC.¹² In a study carried out in Burkina Faso,¹⁸ LBCs were transported in 100% of cases in thermo stable coolers without an "ice-box".

Practitioners of the Blood Transfusion (BT)

As a medical procedure, BT must be performed by the doctor or his/her delegacy to the nurse or midwife and in the absence of any adverse effects.¹⁹ In this study we noticed that most of the LBCs were administered by nurses (70.4%) and then by resuscitator doctors (29.6%). In Togo, Aboubakari's study revealed that 73.2% of LBCs had been transfused by intern trainees, 26.2% by nurses and 0.6% by doctors. Sawadogo noted that all LBCs were transfused by nurses.¹⁸

Type of Blood Transfusion

In the current approach, all transfusions were performed in type-specific (ABO) and cross-matched Rh with homologous blood. This result is similar to the one of Louati²⁰ who noticed that all transfusions of Labile Blood Components were in

cross-matched Rh. Similarly Kouakou et al⁶ reported 98.6% of transfusions in type-specific and 98.6% in cross-matched Rh and with homologous blood in 97.28%. According to the rules of blood transfusion, compatible and identical transfusions (Type-specific and cross-matched Rh) should be preferred over non-identical compatible transfusions (Non-type specific and Non-cross-matched Rh).

Compliance with Transfusion Rules

All the information related to the BT is of a great importance of the acceptance of the patient or her family members. Indeed, before any prescription of a blood transfusion, it is essential to aware the patient or her family members of the necessity for the transfusion. The procedure of transfusion is governed by legislation and engages the responsibility of the prescriber. Any error that may lead to legal proceedings¹². In the current study, no patient or a member of her family was made aware of the disadvantages of transfusion. On the other hand, 70.4% of patients or their companions were informed about the advantages of transfusion. In Burkina Faso, Sawadogo¹⁸ in 2014 noted that the necessity for BT had been explained to patients and/or their companions, although awareness of the disadvantages of TB was not exhaustive.

All non-conformities (absence of transfusion sheet, quantity of blood to be transfused not specified, the rate of transfusion not indicated, the concordance control between the patient and the BCs to be transfused, the compatibility test at the patient's bedside, the close monitoring of blood transfusion during the first 10 minutes) were reported in a high proportions in the current study. This situation is recurrent in many countries with limited resources^{2,6} and reflects a lack of rigor, the urgent context of transfusion and interdisciplinary consultation (clinicians, anesthesiologists and biologists).

In any haemotherapy, the acute transfusion reactions are mainly immunological, often serious and deserve to be prevented in advance. Our survey shows that 75.9% of transfused patients presented a transfusion reaction. This rate is upper to previous studies carried out in Gyneco-Obstetrical in African countries.^{4,6} Transfusion incidents that occurred during transfusions were not recorded in patient's file and were performed by nurses. This justifies the lack of rigorous follow-up of the transfusion procedure by doctors.

Strengths and Weakness of the Study

This study presented strengths but also weakness at a certain degree.

The weakness of this study is inherent to its methodological approach. Indeed, the presence of the principal investigator at all stages of the transfusion chain could have influenced the transfusion practice and therefore biased the results. In addition, the lack of training of those involved in transfusion practice has not made it possible to accurately assess the performance of the various stakeholders and may justify the high proportions of non-compliances in relation to the various studied parameters. The monocentric nature is another weakness of the present study. The results of this study cannot be extrapolated to other hospital institutions (gynecology and obstetrics department) in Kinshasa. Finally, this study did not consider the medium and long-term complications of the patients included in the study.

Nevertheless, the present study is the first to assess the transfusion practice in the Gyneco-Obstetrics environment of the University Hospital of Kinshasa, highlighting the shortcomings of all the practitioners of the transfusion procedure.

Conclusion

This work has shown that the practice of transfusion has real a problem in the Department of Gynecology and Obstetrics of the UHK in terms of the transport of blood components, the consideration of the rules of haemovigilance, and the transfusion procedure.

Continuing education for all the actors involved in transfusion practice and clinical-biologist collaboration are therefore important to ensure blood transfusion under optimal conditions.

Ethical Considerations Ethics Statement

The protocol for this study has been approved by the National Health Ethics Committee (CNES) of the Ministry of Public Health of the Democratic Republic of Congo under number 264/CNES/BN/PMMF/2020 of 02/11/2020 and the written

consent of the patients was obtained. All participants were informed about the purpose of the study and were voluntary participant complies declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work”.

Disclosure

Dr Guy Ndelembo Baina Snr reports grants, personal fees from Cliniques universitaires de Kinshasa, during the conduct of the study. The authors declare no other conflicts of interest.

References

- Andriamandranto HU, Rajaonarison TJ, Rakoto Alison AO, Randriambelomanana JA. blood transfusion in obstetrical practice at the CHU de gynécologie et obstétrique de befelatanana. *Cardiovasc Digit Health J*. 2021;2:4. doi:10.1016/j.cvdhj.2020.11.004
- Zamané H, Dembelé A, Sawadogo KCC, et al. Evaluation de la pratique transfusionnelle en urgence obstetricale et gynecologique au Centre Hospitalier Universitaire Yalgado Ouedraogo, Ouagadougou (Burkina Faso). *Science Et Technique*. 2014;37(1–2):101–109.
- Biswas S, Rengaraj S. Pattern of Blood Transfusion among Women undergoing Caesarean Section in a Tertiary Health Care Centre in South India. *BMC Complement Altern Med*. 2002;2(1):4. doi:10.1186/1472-6882-2-4
- Bonkougou P, Lankoande M, Kinda B. Indications for transfusion and prognosis of women transfused at the Department of Obstetrics and Gynaecology of the Yalgado Ouedraogo University Hospital in Ouagadougou. *Révue Africaine d'Anesthésiologie et de Médecine d'Urgence*. 2014;19(2):18–21.
- Singh S, Sinha P, Yadav G, Gupta U, Tyagi P. Transfusion practices in obstetrics and gynaecology in a tertiary care center. *Int J Reprod Contracept Obstetr Gynecol*. 2017;5(3):831–834. doi:10.18203/2320-1770.ijrcog20160593
- Kouakou F, Effoh D, Loué V, Adjoby R, N'guessan K, Koffi A. Transfusion practice in the gynaeco-obstetrical environment. About 753 transfusions performed at the CHU of Cocody (Abidjan-RCI). *Révue Africaine d'Anesthésiologie et de Médecine d'Urgence*. 2011;16(1):15–19.
- World Health Organization. Regional Office for the Eastern Mediterranean. Strategic Framework for Blood Safety and Availability 2016–2025. World Health Organization. Regional Office for the Eastern Mediterranean; 2016. Available from: <https://apps.who.int/iris/handle/10665/250403>. Accessed June 19, 2022.
- Puri V, Dhal I, Singh K, Sharma G, Rai P, Sharma S. Critical audit of fresh frozen plasma transfusion practices in obstetric and gynecology departments in a tertiary care hospital – where and what needs to be improved? *Glob J Transfus Med*. 2019;4(2):175. doi:10.4103/GJTM.GJTM_49_19
- Chowdhury F, Akhter S, Islam A, Rayen J, Begum N, Begum F. Evaluation of blood transfusion practices in obstetrics and gynecology in a tertiary hospital in Bangladesh. *J Bangladesh Coll Phys Surg*. 2016;34(1):9–14. doi:10.3329/jbcps.v34i1.29116
- Ouh YT, Lee KM, Ahn KH, et al. Predicting peripartum blood transfusion: focusing on pre-pregnancy characteristics. *BMC Pregnancy Childbirth*. 2019;19(1):477. doi:10.1186/s12884-019-2646-3
- Cauldwell M, Shamsheersaz A, Wong TY, et al. Retrospective surveys of obstetric red cell transfusion practice in the UK and USA. *Int J Gynaecol Obstet*. 2017;139(3):342–345. doi:10.1002/ijgo.12309
- Shander A, Gross I, Hill S, Javidrooz M, Sledge S. A new perspective on best transfusion practices. *Blood Transfus*. 2013;11(2):193–202. doi:10.2450/2012.0195-12
- Goubran HA, Elemetry M, Radosevich M, Seghatchian J, El-Ekiaby M, Burnouf T. Impact of transfusion on cancer growth and outcome. *Cancer Growth Metastasis*. 2016;9:1–8. doi:10.4137/CGM.S32797
- Chhabra S, Namgyal A. Rationale use of blood and its components in obstetric-gynecological practice. *J Mahatma Gandhi Inst Med Sci*. 2014;19(2):93. doi:10.4103/0971-9903.138427
- Surbek D, Vial Y, Girard T, et al. Patient blood management (PBM) in pregnancy and childbirth: literature review and expert opinion. *Arch Gynecol Obstet*. 2020;301(2):627–641. doi:10.1007/s00404-019-05374-8
- Bassowa A, Douaguibe B, Ajavon D, Fiagnon K, Aboubakari S, Akpadza K. Satisfaction and time to obtain blood products in obstetric emergency situations at Sylvanus Olympio Teaching University Center of Lome. *Int J Res Rep Gynaecol*. 2020;25:1–8.
- Green L, Knight M, Seeney FM, et al. The epidemiology and outcomes of women with postpartum haemorrhage requiring massive transfusion with eight or more units of red cells: a national cross-sectional study. *BJOG*. 2016;123(13):2164–2170. doi:10.1111/1471-0528.13831
- Sawadogo A. *Blood transfusion practice in the gynaecology and obstetrics department of the Yalgado Ouedraogo University Hospital (Burkina Faso)*. UNIVERSITE DE OUAGADOUGOU; 2012.
- Chand NK, Subramanya HB, Rao GV. Management of patients who refuse blood transfusion. *Indian J Anaesth*. 2014;58(5):658–664. doi:10.4103/0019-5049.144680
- Louati N, Rekik T, Chaabane R, Chaabane K, Mnif H, Gargouri EJ. Evaluation of transfusion practices in obstetrical environment. 2007.

Journal of Blood Medicine**Dovepress****Publish your work in this journal**

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Pegcetacoplan: A New Opportunity for Complement Inhibition in PNH

Ilene Ceil Weitz

To cite this article: Ilene Ceil Weitz (2023) Pegcetacoplan: A New Opportunity for Complement Inhibition in PNH, Journal of Blood Medicine, , 239-245, DOI: [10.2147/JBM.S362220](https://doi.org/10.2147/JBM.S362220)

To link to this article: <https://doi.org/10.2147/JBM.S362220>



© 2023 Weitz.



Published online: 29 Mar 2023.



Submit your article to this journal



Article views: 498



View related articles



View Crossmark data



Citing articles: 7 View citing articles

Pegcetacoplan: A New Opportunity for Complement Inhibition in PNH

Ilene Ceil Weitz 

Jane Anne Nohl Division of Hematology, Keck-USC School of Medicine, Los Angeles, CA, USA

Correspondence: Ilene Ceil Weitz, Jane Anne Nohl Division of Hematology, Keck-USC School of Medicine, Los Angeles, CA, USA, Tel +1-323-865-3950, Fax +1-323-865-0040, Email iweitz@usc.edu

Abstract: Pegcetacoplan is the newest inhibitor of the complement system to be approved by the FDA and EMA for the treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH). The cyclic peptide inhibitor of C3 was evaluated in several clinical trials in PNH leading to its approval. The focus of this paper will review the efficacy and safety of Pegcetacoplan (PEG), and considerations for use in patients with PNH.

Keywords: PNH, complement inhibition, extravascular hemolysis

Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria is a rare hematologic disorder. It is due to an acquired mutation on the X chromosome (PIG-A) which prevents the formation of the glycosylphosphatidylinositol (GPI) anchors on cell membranes. Among the multiple proteins bound to the cell membrane by these GPI anchors, are the complement regulators, CD55, (decay accelerating factor), and CD59, which blocks the insertion of Complement protein 9, the final protein of the membrane attack (MAC). The loss of these anchored complement regulators leads to uncontrolled complement activation on the cell membrane resulting in cellular destruction. This leads to intravascular hemolysis, granulocyte, monocyte and platelet activation leading to thrombosis and cytokine mediated inflammation. PNH is a very morbid disease, limiting the lifespan of the affected patients many of whom are in their 20–30s.^{1–3}

PNH is the best studied of all the complement mediated disorders. The introduction of complement inhibition dramatically altered the morbidity and mortality of PNH and has given us new insights into the role of complement in health and disease. Red cell destruction with intravascular hemolysis is the most obvious manifestation of the effect of complement dysregulation. Hemolysis is continuous but may be exacerbated by increase complement activation, in the setting of infection (both bacterial and viral), vaccination, pregnancy, trauma, and surgery.^{1–3} While more complement activity occurs at night due to exposure of blood to bacterial antigens in the gastrointestinal tract, intravascular hemolysis and hemoglobinuria occurs all time to varying degrees. Paroxysms represent episodes of increased complement activation, red cell destruction and cellular activation.²

Thrombosis, the most dreaded complication of PNH, occurs in up to 60% of patients. Complement activation of platelets, granulocytes, and monocytes contributes to and is the underlying mechanism of the thrombogenicity of the disorder.^{4,5}

PNH is seen in all ages groups but most often presents in patient in their late twenties and early thirties.² Untreated, thirty percent of patients the patients will die within 5 years of diagnosis, half will die by 10 years even with best supportive (BSC).² If the patient has a thromboembolic complication, it is likely they will die within 4 years. Historic treatments such as steroids, and prednisone are anecdotal and ineffective.²

Complement Inhibition

The role of the Complement system in health and disease has become an active area of research and drug development. The development of C5 inhibitors for PNH/atypical hemolytic uremic syndrome (ahus), myasthenia gravis, and neuromyelitis optica spectrum disorder (NMSOD) has revolutionized the treatment of these diseases, improving the quality of life and survivals in these disorders. The most recent additions to this list of approved complement inhibitors are Pegcetacoplan (Empaveli), approved in 2019 for the treatment of PNH and a C1S inhibitor, Sutimlimab, approved in 2021, for the treatment of Cold Agglutinin disease (CAD).

The use of complement inhibition has dramatically changed the outlook for patients with PNH. Eculizumab and ravulizumab (inhibitors of complement protein 5) have altered the morbidity and mortality of the disease by blocking terminal complement activation. The pilot trial, Triumph, Shepherd and AEGIS trials all demonstrated rapid reproducible reduction of hemolysis as measured by lactic dehydrogenase (LDH) and a marked reduction in thrombotic risk, as measured by D-Dimer.^{6–10} As a result, overall mortality has normalized to that of age matched controls. Ravulizumab, the long acting C5 inhibitor, has been shown to be non-inferior to the parent drug, eculizumab, with similar benefits.^{6–10}

However, not all PNH patients respond equally to C5 inhibition. There are rare patients who have a polymorphism in C5, which renders the C5 resistant to eculizumab and ravulizumab.¹¹ About 20–30% of patients achieve hemoglobin normalization, which leaves 70% of patients with some component of anemia.^{12–16} Fifteen to 20% will remain transfusion dependent. Unless there is a significant component of bone marrow failure, most patients have high retic counts with normal LDH consistent with extravascular hemolysis. LDH levels are usually normal or modestly increased (<1.5 times the upper limit of normal) in extravascular hemolysis. Risitano et al noted increased Complement 3b (C3b) loading of RBC due to C5 blockade resulting in extravascular clearance in the spleen and liver due to C3 fragments^{12,13} Extravascular hemolysis is observed in most patients with PNH who are being treated with C5 inhibitors, and can manifest as persistent anemia, reticulocytosis, hyperbilirubinemia and the need for transfusion^{14–16} (Table 1).¹⁴ Complement 3 (C3) inhibition with Pegcetacoplan (APL-2) is designed to block extravascular clearance.

Pegcetacoplan

Pegcetacoplan (PEG) is a cyclic peptide related to compstatin, which blocks C3 preventing the generation of C3b and subsequent C3b loading of the red blood cells (Figure 1).^{2,17,18,20}

Table 1 Proposed Classification of Response to Complement Inhibition in PNH

Response to C5 Inhibition	Need for Transfusion	Hemoglobin (g/dl)	LDH	ARC
Complete	None	>12	< 1.5X UNL	< 150,000
Major	None	>12	>1.5X UNL	>150,000
Good	None	>10 <12	A < 1.5X UNL B >1.5X UNL	r/o bone marrow failure
Partial	None or <2/ 6 mos	>8 <10	A <1.5XUNL B >1.5X UNL	r/o bone marrow failure
Minor	Occasional 50% reduction Regular >3/6 mos	>8 <10 < 10 < 10	A <1.5X UNL B>1.5X UNL	r/o bone marrow failure
No response	Regular >6/6 mos	< 10	A <1.5X UNL B >1.5X UNL	r/o bone marrow failure

Notes: Adapted from Risitano AM, Marotta S, Ricci P et.al. Anti-complement Treatment for Paroxysmal Nocturnal Hemoglobinuria: Time for Proximal Complement Inhibition? A Position Paper From the SAAWP of the EBMT. *Front Immunol*. 2019; 10: 1157.¹⁴

Abbreviations: LDH, lactic dehydrogenase; ARC, absolute reticulocyte response.

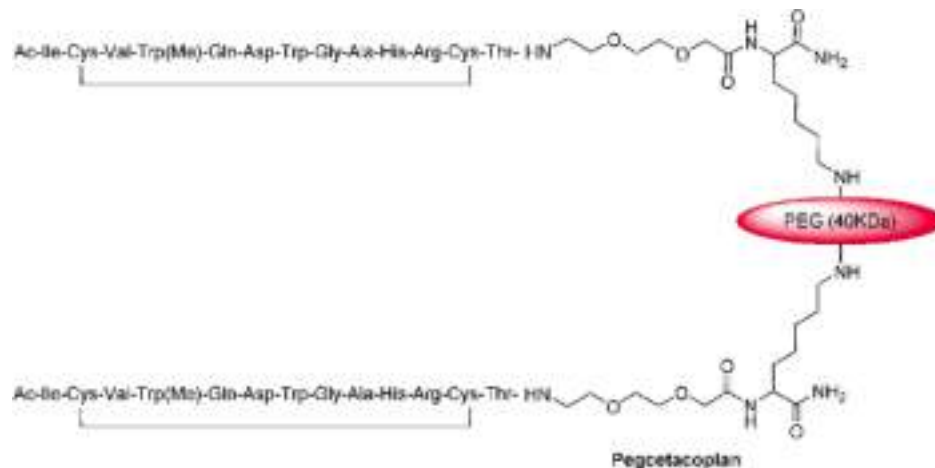


Figure 1 Structure of Pegcetacoplan. Reproduced from Al Shaer D, Al Musaimi O, Albericio F, de la Torre BG. 2021 FDA TIDES (Peptides and Oligonucleotides) Harvest. Pharmaceuticals. 2022; 15(2):222. Creative Commons.²⁸

Pharmacology of Pegcetacoplan

Following a single subcutaneous infusion in healthy subjects, pegcetacoplan was gradually absorbed into the systemic circulation and reached maximum concentrations with a median time of 108–144 h (4.5–6.0 days)^{17,18} In PNH patients treated with pegcetacoplan for sixteen weeks, steady-state serum concentrations were reached in 4–6 weeks after the first dose, with mean steady-state trough serum concentrations ranging from 655 to 706 µg/mL. Exposure to pegcetacoplan increased proportionally over a dose range of 45–1440 mg (0.015 L/h) and 8.0 days, respectively^{17,18} Pegcetacoplan is metabolized by catabolic pathways into small peptides. The drug is broken down into peptides and amino acids and using radiolabeled peptides, the drug was primarily eliminated via urinary excretion.^{17,18}

The estimated bioavailability in patients with PNH is 77%, mean volume of distribution has been measured at ≈ 3.9 L, the mean clearance and median effective elimination half-life of subcutaneous pegcetacoplan, 0.015 L/h, and 8.0 days, respectively.^{17,18}

There may be interference between silica reagents in coagulation panels and pegcetacoplan that results in artificially prolonged activated partial thromboplastin time (aPTT). Therefore, avoid the use of silica reagents in coagulation panels. A chromogenic assay is preferred. Significant prolongation of the QT interval was not observed.

Clinical Studies

The PADDOCK study investigated PEG (APL-2) as monotherapy in untreated PNH demonstrated meaningful reduction in intravascular hemolysis, defined as LDH greater the 1.5 times the upper limit of normal. PEG (APL-2) was given SC as daily infusions, in different dosing cohorts 180 mg (cohort 1) or 270 mg (cohort 2). Reduction of LDH was observed in all patients, with 95% of patients achieving LDH normalization by day 29 of treatment. LDH remained in the normal range at all subsequent time points investigated. Similarly, hemoglobin increased from median 8.0 g/dL at baseline to median 10.8 g/dL at day 29, and 12.2 g/dL when APL-2 treatment was continued to day 85.¹⁹

The PHAROAH trial, a prospective, nonrandomized single and multiple ascending dose phase Ib trial demonstrated efficacy and safety of PEG (APL-2). Patients received 270mg/day. Of the 6 patients in cohort 4 (the highest dose cohort), 4 were able to discontinue their eculizumab with sustained improvement in hemoglobin and LDH on PEG alone.²⁰ Hemoglobin levels were below the LLN in all subjects at baseline (range 7.0–10.5 g/dL). By day 29, all patients had an increase in hemoglobin which remained stable throughout the rest of the study. Two subjects the increase in hemoglobin were high enough to allow for regular phlebotomies as treatment of severe transfusion-related iron overload.²⁰

One patient developed transaminitis necessitating a pause of the APL2 (PEG). After resumption, the transaminases again increased. The patient then underwent a choledochojunostomy with resolution of the transaminitis. After the operation, there was no recurrence of the transaminitis once the PEG was resumed.²⁰

Pegasus

The PEGASUS trial randomized patients with PNH treated with eculizumab but who remained anemic, Hemoglobin < 10, or transfusion dependent were entered into 2 groups. Both groups received PEG + eculizumab for 4 weeks, then randomized to receive PEG alone vs eculizumab (ECU) alone for 16 weeks^{21,22}. The trial demonstrated superiority PEG to eculizumab in the reduction of hemolysis and transfusion avoidance. PEG was superior to the control for the coprimary endpoints of hemoglobin stabilization as well the change from baseline in LDH levels through Week 26 (Figure 2). Hemoglobin stabilization occurred in 85% of PEG patients compared to 0% (n=30) (n=0/18) in ECU arm at the treatment assessments point, week 26 (difference, 73.1%; 95% CI, 57.2%, 89.0%; P<0.0001). PEG treated patients demonstrated greater improvements from baseline in LDH levels compared to the ECU at Week 26; the least-square (LS) mean (SE) change from baseline in LDH levels at Week 26 in the PEG arm was -1870.5 U/L (101.0) versus -400.1 U/L (313.0) in the control arm (difference, -1470.4 U/L; 95% CI, -2113.4, -827.3; P<0.0001).^{21,22} The side effect profile was similar to that seen in PADDOCK and PHAROAH. The PEGASUS 48-week data demonstrate a durable effect of PEG on hemolysis as measured by hemoglobin, reduction of LDH, reticulocytes and bilirubin. The favorable safety profile, as well as quality of life outcomes, such as FACIT-fatigue score and transfusion avoidance, were sustained through 48 weeks. Patients in the Eculizumab-to-Pegcetacoplan group demonstrated significantly improved hemoglobin levels at 48 weeks (11.45 g/dL [SD, 2.14]) as compared to 16 weeks of treatment (ecu alone) (8.58 g/dL [SD, 0.96]; nominal p-value<0.0001). There was no significant change in Pegcetacoplan's safety profile at 48 weeks as compared with reported data at 16 weeks.²² In a recent sub-analysis of PEGASUS trial, patients with AA-PNH on PEG had durable treatment benefits in all efficacy parameters at week 48, demonstrating outcomes comparable to patients with no history of AA. PEG safety profile was consistent with previous data at 16 weeks.²³

Prince

Can patients start Pegcetacoplan without initial C5 inhibitor treatment? The PRINCE study looked at PNH patients without access to Eculizumab or Ravulizumab.²⁴ The Phase 3, randomized, multicenter (22 sites), open-label, controlled PRINCE study enrolled 53 adult complement-inhibitor naïve patients with PNH. Patients were randomized and stratified based on number of transfusions (<4 or ≥4) within 12 months before screening. Patients were assigned at a 2:1 ratio to receive pegcetacoplan (1080 mg subcutaneously twice-weekly [N=35]) or continue best supportive care (BSC) without

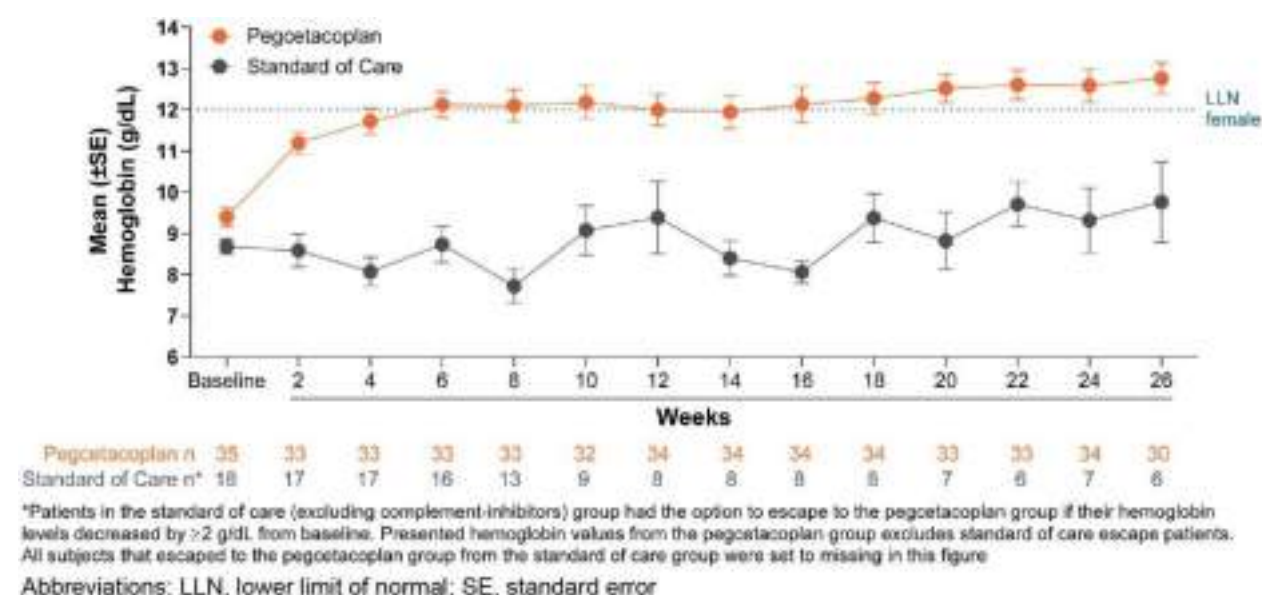


Figure 2 Prince: Observed hemoglobin over time. Reprinted from *Blood*, 138(suppl 1), Wong RS, Navarro JR, Comia NS, et al. Efficacy and safety of pegcetacoplan treatment in complement-inhibitor naïve patients with paroxysmal nocturnal hemoglobinuria: results from the phase 3 prince study. 606, Copyright 2021, with permission from Elsevier.²⁴

complement-inhibitors (control [N=18]) through the 26-week. Coprimary endpoints were hemoglobin stabilization (avoidance of >1 g/dL decrease in hemoglobin levels without transfusions) from baseline through Week 26 and change from baseline in lactate dehydrogenase (LDH) levels at Week 26. Secondary endpoints evaluated additional hematologic parameters, transfusion requirements, fatigue, and quality of life. Safety was analyzed in all patients. Data presented, as an abstract, revealed PEG was superior to SOC in both co-primary endpoints. Hemoglobin stabilization was achieved by 85.7% (n=30) of PEG-treated patients and 0.0% of SOC patients through Week 26 ($p<0.0001$). PEG-treated patients demonstrated superior reductions in mean LDH levels from baseline to Week 26 compared to SOC patients (least-squares mean change from baseline (CFB): PEG, -1870.5 U/L; SOC, -400.1 U/L; $p<0.0001$), and mean LDH levels in PEG-treated patients at Week 26 (mean level: 204.6 U/L) were below the ULN for LDH (226.0 U/L). PEG was also superior to SOC in the secondary endpoints: mean CFB in Hb levels (least-squares mean CFB: PEG, 2.9 g/dL; SOC, 0.3 g/dL; $p=0.0019$; Week 26 mean Hb: PEG, 12.8 g/dL; SOC, 9.8 g/dL) (Figure 1) and transfusion avoidance (PEG, 91.4%, n=32; SOC, 5.6%, n=1; $p<0.0001$). Serious AEs were reported by 8.7% (n=4) of PEG-treated patients and 16.7% (n=3) of SOC patients through Week 26. Two deaths occurred, one in each arm, and were both deemed unrelated to treatment (PEG, 2.9%, n=1, septic shock related to medullary aplasia; SOC, 5.6%, n=1, respiratory failure). No events of meningitis or thrombosis were reported in either group. The most common AEs reported during the study were injection site reaction (PEG, 30.4%, n=14; SOC, 0.0%), hypokalemia (PEG, 13.0%, n=6; SOC, 11.1%, n=2), and fever (PEG, 8.7%, n=4; SOC, 0.0%). None of the AEs led to discontinuation of PEG. It appears that patients with PNH, naïve to complement-inhibitor treatment demonstrated meaningful hematological and clinical improvements following 26 weeks of PEG treatment. The treatment was well tolerated and showed a safety profile similar to those seen in other Pegcetacoplan trials.^{24,25} However, results of this trial remain unpublished at this time.

No thromboembolic events were noted. In the PRINCE study, concomitant use of antithrombotic treatment decreased from 21% (n=11/53) before study entry, to 7% (n=3/46) in pegcetacoplan patients. No change in the number of patients on antithrombotic therapy was observed in the control patients during the study. Two events occurred in the open-label extension, but were deemed unrelated to study drug, a case of Hodgkin's lymphoma, and one case of sepsis. D-dimer normalization rates in PEGASUS PEG arm were similar to those in who stayed on Eculizumab. In the PRINCE study, dimer normalization increased from 51% to 67% by week 8 and further increased to 68% by week 26.²⁶ Only 18% of patients in the control arm of patients (n=2/11) who escaped to PEG had normalized D-dimer levels at baseline, which increased to 27% (n=3/11) at their last lab reading before switching to PEG. After switching to PEG, 82% (n=9/11) had D-dimer normalization at Week 26. PEG treatment improved D-dimer normalization and reduced the incidence of thrombotic events in patients who were complement-inhibitor naïve or remained anemic after stable eculizumab treatment (≥ 3 -months). This suggests that PEG treatment may inhibit complement mediated hemostatic activation, similar to the effect seen with C5 inhibition.^{5,24}

Side Effects

Injection site reactions were the most common side effect in all of trials. However, they were mild and decreased over time, most likely due to increasing patient skill. In the PEGASUS trial, infections occurred in 29% of patients on the PEG arm and 26% in the Eculizumab arm. Diarrhea did occur more frequently in the PEG arm vs the ECU arm (22% vs 3%) but was mild. No grade 3 or 4 events were reported. Hemolysis, as a side effect, was more common in the ECU arm vs PEG (23% vs 10%).^{22,23}

Because of the effectiveness of PEG, PNH clone size on treatment approaches 98%. A concern expressed by Luzzato et al, is that breakthrough hemolysis with PEG is more profound because of the increased clone size. Several cases of severe, life-threatening breakthrough hemolysis have been reported with LDH values of 10–15 times patient baseline. In the clinical trials, fifteen percent of patients discontinued treatment due to breakthrough hemolysis. This may have been due to constraints of the clinical trial which limited the amount of rescue medications. PEG half-life is short so compliance and adherence to the dosing regimen is crucial.²⁷ A maximally effective regimen for breakthrough should be established.

Patient Selection

PEG was broadly approved for the treatment of PNH. Based on the clinical trial data, patients with an inadequate response to C5 inhibition should be considered as candidates for PEG. This includes the rare C5 polymorphism described by Nishimura.¹¹ More often, patients accumulate C3b on the RBC membrane due to inhibition of C5.^{12–16} This leads to opsonization by the red cells by splenic and liver macrophages. It is important to document the presence of C3b on the RBC via a Coombs test. It is also important to have an adequate reticulocyte count. A poor reticulocyte count may be evidence of coincident bone marrow failure (Aplastic Anemia) which is common in PNH. Since the complement cascade is not involved in immune-mediated bone marrow failure, changing the anti-complement treatment is not likely to improve underlying bone marrow failure.

For patients who prefer to do their own treatment, PEG affords an opportunity for self-administration and convenience.

Based on the preliminary PRINCE data, it appears that naïve PNH patients can be treated upfront with PEG with improvement in hemolysis, anemia and a decrease in thromboembolic events. However, at this time, the trial manuscript is pending peer evaluation.

There are no formal guidelines concerning monitoring the effect of Pegcetacoplan. Hemoglobin response, bilirubin, reticulocyte count, and LDH are used. Total hemolytic complement (CH50) does not accurately monitor the alternative complement pathway inhibition. AH50 can be used but may not be readily available.

Trials with Pegcetacoplan that are underway in thrombotic microangiopathy associated with following hematopoietic stem cell transplant (HSCT), C3Glomerulonephritis (C3GN)-Immune complex GN (ICGN), post-transplant C3GN and ICGN and cold agglutinin disease as well as intravitreal treatment for macular degeneration (geographic atrophy).

Funding

No honoraria were paid for this review.

Disclosure

The author reports speaker honoraria, consultant from Alexion Pharmaceutical; she is also a consultant for Apellis Pharmaceuticals, Novartis Pharmaceutical, and Biocryst Pharmaceutical.

References

1. Zipfel PF, Hallstrom T, Riesbeck K. Human Complement Control and complement evasion by pathogenic microbes- Tipping the balance. *Mol Immunol*. 2013;56:152–160.
2. de la Torre BG, Albericio F, Albericio F the pharmaceutical industry in 2021. an analysis of FDA Drug approvals from the perspective of molecules. *Molecules*. 2022;27:1075. doi:10.3390/molecules27031075
3. Hillmen P, Lewis SM, Bessler M, Luzatto L, Dacie J. The natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 1995;333:1253–1258. doi:10.1056/NEJM199511093331904
4. Hill A, Kelly R, Hillmen P. Thrombosis in PNH. *Blood*. 2013;121(25):4985–4996. doi:10.1182/blood-2012-09-311381
5. Weitz IC, Ghods M, Rochanda L, et al. Eculizumab therapy results in sustained reduction in markers of hemostatic activation and inflammation and thrombocytopenia in patients with PNH. *Thromb Res*. 2012;2012:107. PMID: 22542362.
6. Brodsky RA, Young NS, Antonoli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;111:1840–1847. doi:10.1182/blood-2007-06-094136
7. Kelly RJ, Hill A, Arnold LM, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood*. 2011;117:6786–6792. doi:10.1182/blood-2011-02-333997
8. Loschi M, Porcher R, Barraco F, et al. Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: a treatment versus no-treatment study. *Am J Hematol*. 2016;91:366–370. doi:10.1002/ajh.24278
9. Lee JW, Kulasekararaj AG. Ravulizumab for the treatment of paroxysmal Nocturnal Hemoglobinuria. *Expert Opin Biol Ther*. 2020;20(3):227–233. doi:10.1080/14712598.2020.1725468
10. Kulasekararaj AG, Hill A, Langemeijer S, et al. One-year outcomes from a phase 3 randomized trial of ravulizumab in adults with paroxysmal nocturnal hemoglobinuria who received prior eculizumab. *Eur J Haematol*. 2021;106(3):389–397. doi:10.1111/ejh.13564PMCID:PMC8246907
11. Nishimura J, Yamamoto M, Hayashi S, et al. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med*. 2014;370(7):632–639. PMID: 24521109. doi:10.1056/NEJMoa1311084
12. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. *Blood*. 2009;113:4094–4100. doi:10.1182/blood-2008-11-189944.13

13. Hill A, Rother RP, Arnold L, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. *Haematologica*. 2010;95:567–573. doi:10.3324/haematol.2009.007229
14. Risitano AM, Marotta S, Ricci P, et al. Anti-complement treatment for paroxysmal nocturnal hemoglobinuria: time for proximal complement inhibition? A position paper from the SAAWP of the EBMT. *Front Immunol*. 2019;10:1157. doi:10.3389/fimmu.2019.01157
15. Risitano AM. Paroxysmal nocturnal hemoglobinuria and the complement system: recent insights and novel anticomplement strategies. *Adv Exp Med Biol*. 2013;735:155–172. doi:10.1007/978-1-4614-4118-2_10
16. Risitano AM, Notaro R, Luzzatto L, et al. Paroxysmal nocturnal hemoglobinuria--hemolysis before and after eculizumab. *N Engl J Med*. 2010;363:2270–2272. doi:10.1056/NEJMc1010351
17. Apellis Pharmaceuticals Inc. EMPAVELITM (pegcetacoplan) injection, for subcutaneous use; 2021. Available from: <https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=c23d89e9-b00b-4520-e053-2995a90a95af>. Accessed September 5, 2022.
18. Swedish Orphan Biovitrum AB (publ). ASPAVELI 1 080 mg solution for infusion; 2021. Available from: <https://www.ema.europa.eu/en/medicines/human/EPAR/aspaveli>. Accessed September 5, 2022.
19. Wong RSM, Pullon HWH, Deschatelets P, et al. Inhibition of C3 with APL-2 results in normalisation of markers of intravascular and extravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood*. 2018;132:2314. doi:10.1182/blood-2018-99-110827
20. De Castro C, Grossi F, Weitz IC, et al. C3 inhibition with pegcetacoplan in subjects with paroxysmal nocturnal hemoglobinuria treated with eculizumab. *Am J Hematology*. 2020;95(11):1334–1343. doi:10.1002/ajh.25960
21. Peter Hillmen P, Szer J, Weitz IC, et al. Pegcetacoplan versus eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2021;394:1028–1037. doi:10.1056/NEJMoa2029073
22. De la Tour RP, Szer J, Weitz IC, et al. Pegcetacoplan versus eculizumab in patients with paroxysmal nocturnal hemoglobinuria (PEGASUS): 48 week follow-up of a randomised open label phase 3 active comparator controlled trial. *Lancet Haematol*. 2022;e2022(9):e648–659.
23. Usuki K. The evaluation of pegcetacoplan in paroxysmal nocturnal haemoglobinuria patients with aplastic anemia. 2nd annual ESH-EBMT translational conference; 2022.
24. Wong RS, Navarro JR, Comia NS, et al. Efficacy and safety of pegcetacoplan treatment in complement-inhibitor naïve patients with paroxysmal nocturnal hemoglobinuria: results from the phase 3 prince study. *Blood*. 2021;138(suppl):606. doi:10.1182/blood-2021-147493
25. Ueda Y, Takamori H, Nishimura JI. Pegcetacoplan versus Eculizumab in PNH. *N Engl J Med*. 2021;385(18):1723–1724. PMID: 34706182.
26. Weitz IC, Al-Adhami M, Min J, et al. Patients with Paroxysmal Nocturnal Hemoglobinuria Treated with Pegcetacoplan Show Improvements in D-dimer Normalization and Decrease in Incidence of Thrombosis EHA; 2022.
27. Notaro R, Luzatto L, Notaro R. Breakthrough hemolysis in PNH with proximal or terminal complement inhibition. *NEJM*. 2022;387:2. doi:10.1056/NEJMra2201664
28. Al Shaer D, Al Musaimi O, Albericio F, de la Torre BG. 2021 FDA TIDES (peptides and oligonucleotides) harvest. *Pharmaceuticals*. 2022;15(2):222. doi:10.3390/ph15020222

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Howell-Jolly Body-Like Inclusions in Coronavirus Disease 2019 (COVID-19): Possible Novel Findings

Amaylia Oehadian, Ian Huang, Andini Kartikasari, Bachti Alisjahbana & Delita Prihatni

To cite this article: Amaylia Oehadian, Ian Huang, Andini Kartikasari, Bachti Alisjahbana & Delita Prihatni (2023) Howell-Jolly Body-Like Inclusions in Coronavirus Disease 2019 (COVID-19): Possible Novel Findings, Journal of Blood Medicine, , 233-238, DOI: [10.2147/JBM.S399596](https://doi.org/10.2147/JBM.S399596)

To link to this article: <https://doi.org/10.2147/JBM.S399596>



© 2023 Oehadian et al.



Published online: 29 Mar 2023.



Submit your article to this journal



Article views: 345



View related articles



View Crossmark data

Howell-Jolly Body-Like Inclusions in Coronavirus Disease 2019 (COVID-19): Possible Novel Findings

Amaylia Oehadian¹, Ian Huang^{2,3}, Andini Kartikasari⁴, Bakti Alisjahbana⁵, Delita Prihatni⁶

¹Division of Hematology and Oncology Medic, Department of Internal Medicine Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia; ²Department of Internal Medicine Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia; ³Faculty of Medicine, Universitas Pelita Harapan, Tangerang, Banten, Indonesia; ⁴Bandung City Regional General Hospital, Bandung, Indonesia; ⁵Division of Infectious and Tropical Diseases, Department of Internal Medicine Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia; ⁶Department of Clinical Pathology Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

Correspondence: Amaylia Oehadian, Department of Internal Medicine, Padjadjaran University/Dr. Hasan Sadikin General Hospital, Jl. Pasteur No. 38, Bandung, West Java, 40161, Indonesia, Email amaylia.oehadian@unpad.ac.id

Background: During COVID-19 pandemic, it is difficult to distinguish febrile patient infected by SARS-CoV-2 or bacterial causes. Howell-Jolly bodies are a well-known entity found in red blood cells. They are nuclear fragments, composed of deoxyribonucleic acid, commonly observed in the peripheral blood smears of hyposplenic or asplenic patients. Recently, similar inclusions often referred to as Howell-Jolly body-like inclusions (HJBLIs) have been reported in the neutrophils of patients with acquired immune deficiency syndrome (AIDS) and COVID-19 patient.

Aim: To explore whether HJBLIs in peripheral blood smear could differentiate between patients with confirmed SARS-CoV-2 and bacterial pneumonia.

Methods: We performed cross-sectional study using secondary data from COVID-19 database and re-evaluated peripheral blood smears to identify HJBLIs. We included confirmed COVID-19 adults age ≥ 18 years who were hospitalized in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia from March 1st 2020–May 31st 2020. We also examined peripheral blood smears in patients with confirmed bacterial pneumonia as a control group. Clinical characteristics including disease severity, CURB-65 score, comorbidity, and the present of HJBLIs in peripheral blood smears were evaluated.

Results: Overall, 33 patients were included: 22 were confirmed COVID-19 and 11 were confirmed bacterial pneumonia. The median (interquartile range) age in COVID-19 and patients with bacterial pneumonia were 53 years (40–64) vs 57 years (53–71), respectively. Compared with patients with bacterial pneumonia, HJBLIs were significantly higher in COVID-19 patients [21/22 (80.8%) vs 5/11 (45.5%), $p = 0.001$].

Conclusion: Howell-Jolly body-like inclusions could be a potential feature to help differentiate between COVID-19 and bacterial pneumonia.

Keywords: Howell-Jolly body-like inclusions, COVID-19, bacterial pneumonia, peripheral blood smear

Introduction

During COVID-19 pandemic many patients with acute febrile illness were hospitalized. It was difficult to distinguish between patients with SARS-CoV-2 and those who have bacterial pneumonia. Diagnostic laboratory confirmation such as polymerase-chain reaction for COVID-19 and bacterial culture need time and specific resources that were not available in every health facility. Especially in severe patients, we need to early differentiate between COVID-19 and bacterial pneumonia patients to determine the appropriate treatment.

The peripheral blood smear is a simple evaluation that can potentially differentiate between bacterial and viral infection.¹ One of the abnormalities that could be detected in peripheral blood smear is Howell-Jolly bodies, nuclear remnants found in red blood cells under various pathological states. They are most commonly present in patients with

absent or impaired function of the spleen² Others causes of Howell-Jolly bodies are post-splenectomy, sepsis, post-high-dose corticosteroid treatment, autoimmune and neoplastic disorders.^{3,4}

In peripheral blood smears, the nuclear fragments appear as small but strongly basophilic (purple) dots inside the eosinophilic (pink) red blood cells.⁵ Howell-Jolly body-like inclusions (HJBLIs) were detached nuclear fragments resembling Howell-Jolly bodies within neutrophils from Human Immunodeficiency Virus (HIV)-infected patients, organ-transplant recipients, and patients on immunosuppressive drugs.⁶ In our previous case report, interestingly we found HJBLIs in our COVID-19 patient.⁷

In this study, we sought to determine whether the presence of HJBLIs could be used to help differentiating between patients with confirmed COVID-19 and bacterial pneumonia.

Methods

We performed cross-sectional study using secondary data from COVID-19 database and re-evaluated peripheral blood smears to identify HJBLIs. We included confirmed COVID-19 adults age ≥ 18 years who were hospitalized in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia from March 1st 2020–May 31st 2020. We also examined peripheral blood smears in patients with suspected COVID-19 but had negative SARS-CoV-2 RT-PCR and confirmed bacterial pneumonia in that period as a control group. From this database, we excluded the patients who had not complete data of hematological examination and peripheral blood smears. Clinical characteristics including disease severity, CURB-65 scoring, comorbidity, and the present of HJBLIs in peripheral blood smears were compared on the hospital admission. Disease severity was determined using WHO interim guidelines of novel coronavirus (2019-nCoV) (WHO 2020).⁸

We retrieved complete blood cell counts (CBCs) data that were performed using Sysmex XN-1000. Peripheral blood smears were re-evaluated manually by two independent observers, a board-certified haematologist and a hematopathologist. The present of HJBLIs, morphological characteristics in red blood cells, white blood cells (WBC), and platelets were qualitatively and quantitatively documented. The morphology grading system was reported according to the International Council for Standardization in Hematology (ICSH), and the examiners were blinded to the COVID-19 test result during the smear review.

Qualitative variables of peripheral blood smear were analysed using the Chi-square or Fisher's Exact test, while the Mann-Whitney test was used to conduct quantitative analysis. All statistical analyses were conducted using SPSS software (SPSS Inc. 24).

Results

A total of 33 patients which consist of 22 COVID-19 infected cases and 11 bacteria pneumonia cases were included in the analysis. More than half of patients were male. Median (IQR) age of COVID-19 and bacterial pneumonia patients were 53 years (40–64) vs 57 years (53–71) years, respectively. The most common comorbidity was hypertension in patient with COVID-19 (40.9%), and chronic kidney disease in patients with bacterial pneumonia (36.4%). Severe disease was found in approximately half of COVID-19 and two-third of bacterial pneumonia patients. CURB-65 score 3 (severe pneumonia) was significantly higher in bacterial pneumonia compared to COVID-19 patients [3/11 (27.3%) vs 0/22 (0%), $p = 0.03$]. The majority of our COVID-19 and bacterial pneumonia patients were discharged. Death was found in approximately 14% COVID-19 patients and one-fourth of those with bacterial pneumonia (Table 1).

Haemoglobin level was higher in COVID-19 patients compared to those with bacterial pneumonia [12.5 (6.4–12.8) vs 9.5 (7.2–12.6) g/dL, $p = 0.004$]. There were no significant differences in white blood cells count and morphology in peripheral blood between COVID-19 and bacterial pneumonia patients, except for HJBLIs. Proportion of COVID-19 patients that had HJBLIs was significantly higher than those with bacterial pneumonia [21/22 (80.8%) vs 5/11 (45.5%), $p = 0.002$]. Atypical lymphocytes were commonly found in COVID-19 patients [9/22 (40.9%)]. There was no COVID-19 patient with thrombocytopenia compared to half of those with bacterial pneumonia patients ($p = 0.001$) (Table 2).

Howell-Jolly body-like inclusions are depicted in Figure 1.

Table 1 Characteristics of Patients with COVID-19 and Bacterial Pneumonia

Characteristics	COVID-19 (n= 22)	Bacterial Pneumonia (n= 11)
Age, years (median, IQR)	53 (40–64)	57 (53–71)
Male (n, %)	12 (54.5)	7 (63.6)
Comorbidity (n, %) ^a		
• Diabetes	5 (22.7)	0 (0.0)
• Hypertension	9 (40.9)	3 (27.3)
• Coronary artery disease	3 (13.6)	2 (18.2)
• Chronic kidney disease	0 (0.0)	4 (36.4)
• Tuberculosis	0 (0.0)	2 (18.2)
• Cirrhosis of the liver	0 (0.0)	1 (9.1)
• Others	7 (63.6)	5 (22.7)
Severity of disease (n, %)		
• Moderate	10 (45.5)	3 (27.3)
• Severe	12 (54.5)	8 (72.7)
CURB-65 score (n, %) ^b		
• 0	2 (9.1)	1 (9.1)
• 1	16 (72.7)	4 (36.4)
• 2	4 (18.2)	3 (27.3)
• 3 ^c	0 (0.0)	3 (27.3)
Bacterial sputum culture	No bacteria	E. Coli: 4 (36.4) Acinetobacter baumannii: 3 (27.2) Staphylococcus hemolyticus: 3 (27.2) Klebsiella pneumonia: 1 (9.1)
Outcome (n, %)		
• Death	3 (13.6)	3 (27.3)
• Discharged	19 (86.4)	8 (72.7)

Notes: ^aPatients may have more than one comorbidity. ^bp = 0.038. ^cp = 0.03.

Table 2 Red Blood Cells, White Blood Cells and Platelets in Patient with COVID-19 and Bacterial Pneumonia

Variable	COVID-19 (n=22)	Bacterial Pneumonia (n=11)	p-value
Haemoglobin (g/dL), median (IQR)	12.5 (6.4–12.8)	9.5 (7.2–12.6)	0.004^a
White blood cells (/mm ³), median (IQR)	12,500 (3120–21,000)	10,610 (4920–20,610)	0.194
Platelets (x1000/mm ³), median (IQR)	257 (64–633)	109 (42–419)	0.24
Peripheral blood smear Red blood cells			
Size			
Normocytic (n, %)	1 (4.5)	0 (0.0)	1.000
Anisocytosis (n, %)	21 (95.5)	11 (100.0)	
Chromic			
Normochromic (n, %)	7 (31.8)	0 (0.0)	0.040^b
Polychromatic (n, %)	15 (68.2)	11 (100.0)	
Shape			
Burr Cell (n, %)	9 (40.9)	6 (54.4)	0.458
Schistocyte (n, %)	12 (54.5)	2 (18.2)	0.124
Target Cell (n, %)	15 (68.2)	4 (36.4)	0.086
Ovalocyte (n, %)	0 (0.0)	0 (0.0)	-
Spherocyte (n, %)	14 (63.6)	9 (81.8)	0.256
Normoblast (n, %)	2 (9.1)	0 (0.0)	0.438

(Continued)

Table 2 (Continued).

Variable	COVID-19 (n=22)	Bacterial Pneumonia (n=11)	p-value
White blood cells			
Amount			
Decrease (n, %)	4 (18.2)	1 (9.1)	0.383
Normal (n, %)	17 (77.3)	8 (72.7)	
Increase (n, %)	1 (4.5)	2 (18.2)	
Immature cells			
Myelocyte (n, %)	7 (31.8)	2 (18.2)	0.347
Metamyelocyte (n, %)	1 (4.5)	0 (0.0)	0.667
Hypersegmentation (n, %)	8 (36.4)	4 (36.4)	0.653
Toxic Granule (n, %)	10 (45.5)	6 (54.5)	0.622
Atypical Lymphocytes (n, %)	9 (40.9)	2 (18.2)	0.182
Vacuole			
Neutrophil (n, %)	4 (18.2)	1 (9.1)	0.450
Monocyte (n, %)	18 (81.8)	8 (72.7)	0.429
Howell-Jolly body like inclusion (n, %)	21 (80.8)	5 (45.5)	0.002^b
Platelets amount			
Decrease (n, %)	0 (0.0)	6 (54.5)	0.001^c
Normal (n, %)	20 (90.9)	4 (36.4)	
Increase (n, %)	2 (9.1)	1 (9.1)	
Giant platelets (n, %)	19 (86.4)	11 (100.0)	0.282

Notes: ^aMann-Whitney, ^bChi Square, ^cFisher Exact test.

Discussion

This study demonstrated significant morphologic changes between patients with and without COVID-19 infection. Our most interesting findings are the significant HJBLIs found in these patients. COVID-19 patient also showed normal platelet, and a slight increase in atypical lymphocytes.

The findings of HJBLIs in neutrophils were previously reported in COVID-19 patient.⁷ And observed in majority of our patients. Bain coined the inclusion in 1989, a detached nuclear fragment in the neutrophils caused by dysplastic granulopoiesis.⁹ It was occasionally identified in monocytes, lymphocytes, eosinophils, and neutrophils.¹⁰ The occurrence of the inclusions could hypothetically be a response to a specific viral infection. However, the pathogenic and prognostic consequences of HJBLIs in COVID-19 are questions that demand further studies. We also found HJBLIs in 5/

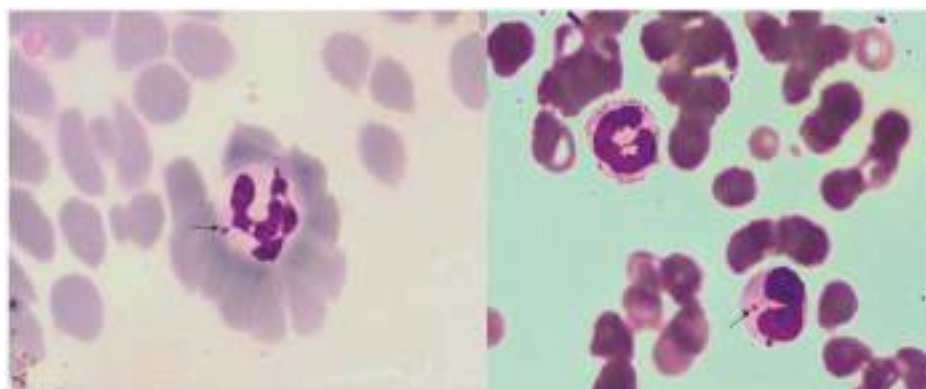


Figure 1 Howell-Jolly body-like inclusions found in neutrophil (arrow sign).

11 (45.5%) patients with bacterial infected group due to possibility previous or concomitant other viral infection in these patients.

Interestingly and different from other common viral infections, about 90% of the COVID-19 patients had normal platelet count. This finding is also consistent with the previous study by Pozdnyakova, which reported normal mean platelet count in Non-ICU and ICU COVID-19 patients.¹¹ Infected patients often have mild thrombocytopenia and increased platelet consumption, together with a corresponding increase in platelet production.¹² On the contrary, we found approximately half of bacterial pneumonia patients had thrombocytopenia. Elmaraghy et al revealed the association of thrombocytopenia with high number of mortality in patient with pneumonia.¹³ Compared to patients with severe pneumonia but without COVID-19, those with COVID-19 disease actually had higher platelet counts, according to Yin et al.¹⁴

In our study, atypical lymphocytes were found in 9/22 (40.9%) COVID-19 patients compared to 2/11 (18.2%) in those with bacterial pneumonia. This finding was lower than previously study by Weinberg et al who reported atypical lymphocytes in 93.3% COVID-19 cases in Chicago, USA.¹⁵ This variance could be caused by the difference in clinical characteristics of the patients. Most of the patients (9/15) in previous study were admitted in intensive care with severe disease compared to half of our patients with severe COVID-19 disease. These atypical lymphocytes are likely reactive to severe SARS-CoV-2 infection.

We found that haemoglobin level was significantly higher in COVID 19 patients compare to bacterial infected cases [12.5 (6.4–12.8) vs 9.5 (7.2–12.6) g/dl, $p = 0.004$]. Previous study reported that patients with severe COVID-19 had significantly lower hemoglobin levels than those with moderate or mild COVID-19. The optimal hemoglobin cut-off value for prediction of disease severity was 11.6 g/dL.¹⁶ These different could be caused by different severity between COVID-19 and bacterial pneumonia patients in our study. On the other hand, there was no significant difference in WBC and platelet count between the groups. However, Kamel et al revealed COVID-19 cases showed a significant decrease in mean WBC count, as well as basophil, neutrophil, lymphocyte, and eosinophil counts compared with the controls.¹⁷ This dissimilarity could be caused by different characteristics of bacterial pneumonia patients in our study.

In regard of clinical characteristics, the most common comorbidity in COVID-19 patient was hypertension followed by diabetes. These results were consistent with a meta-analysis study on COVID-19 comorbidities, in total of 1786 patients that stated the most common comorbidities identified in these patients were hypertension (15.8%), cardiovascular and cerebrovascular conditions (11.7%), and diabetes (9.4%).¹⁸ In bacterial pneumonia patients, the most common comorbidity was chronic kidney disease followed by hypertension. HJBLIs previously reported in HIV-infected patients, organ-transplant recipients, and patients on immunosuppressive drugs.⁶ So, we assumed that the comorbidities in our patients did not affect the present of HJBLIs.

The majority of our COVID-19 patients were discharged and approximately 14% ended in death. These results were consistent with previous study by Chen et al that found discharged and death of COVID-19 patients were 68% and 15%, respectively.¹⁹

The limitations of this study are the small number of subjects; hence, analysis cannot be conducted according to the severity of the disease, and the possibility of operator-dependent morphologic changes in blood smear. We also could not retrieve list of drugs used in our patients, so we could not rule out the potential bias caused by drugs especially steroid use that could have influence on the present of HJBLIs. Post high-dose steroid treatment had been reported as one of the causes of Howell-Jolly bodies in red blood cells.^{3,4} It is unknown whether steroid used could influence HJBLIs. In addition, due to cross-sectional method, we could not evaluate the relation between these new findings in the peripheral blood smear with the course of the patients. In conclusion, blood smear analysis could be considered as an additional important examination in patients with suspected COVID-19 to help differentiate those from bacterial pneumonia. In addition, attention should be focused on HJBLIs in neutrophils. Further studies are needed to evaluate the pathogenic and prognostic consequences of HJBLIs in COVID-19 patients with analysis of additional variables among the groups and stratification by disease severity.

Ethical Approval

Ethical approval was obtained from Ethics Committee of Health Research Dr Hasan Sadikin Hospital, (institution name: RSUP Dr Hasan Sadikin) with following number: LB.02.01/X.6.5/100/2020. We confirm that informed consent was obtained from the study participants. We confirm that the guidelines outlined in the Declaration of Helsinki were followed.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Prokocimer M, Potasman I. The added value of peripheral blood cell morphology in the diagnosis and management of infectious diseases—part 1: basic concepts. *Postgrad Med J*. 2008;84(997):579–585. doi:10.1136/pgmj.2008.069609
2. Sears DA, Udden MM. Howell-Jolly bodies: a brief historical review. *Am J Med Sci*. 2012;343(5):407–409. doi:10.1097/MAJ.0b013e31823020d1
3. William BM, Corazza GR. Hyposplenism: a comprehensive review. Part I: basic concepts and causes. *Hematology*. 2007;12(1):1–13.
4. Ong SY, Ng HJ. Howell-Jolly bodies in systemic amyloidosis. *Int J Hematol*. 2018;108(2):119–120. doi:10.1007/s12185-018-2473-8
5. Mathew H, Dittus C, Malek A, Negroiu A. Howell-Jolly bodies on peripheral smear leading to the diagnosis of congenital hyposplenism in a patient with septic shock. *Clin Case Rep*. 2015;3(8):714. doi:10.1002/ccr3.323
6. Chang B, Huang RSP, Dasgupta A, Nguyen N, Wahed A. Prevalence of Howell-Jolly body-like inclusions in HIV patients and their correlation with CD4 counts and HIV RNA viral load. *Ann Clin Lab Sci*. 2015;45(1):23–26.
7. Alisjahbana B, Huang I, Oehadian A. The detection of Howell-Jolly body-like inclusions in a case of coronavirus disease-2019 (COVID-19). *Blood Res*. 2020;55(4):191. doi:10.5045/br.2020.2020186
8. Koçyiğit B, Sayin S, Şişman FO, Kizilkaya S, Ayrik MY. Coronavirus disease-19 (Covid-19): the disease that changed the world. *Turkish Med Student J*. 2020;7(2):96–105. doi:10.4274/tmsj.galenos.2020.07.02.07
9. Bain BJ. *Blood Cells: A Practical Guide*. Philadelphia: Lippincott; 1989.
10. Andre E, Chevalier C, Scheiff JM. Howell-Jolly-like bodies in leucocytes: first description in leucocytes other than neutrophils. *Eur J Haematol*. 2011;86(2):182–183. doi:10.1111/j.1600-0609.2010.01543.x
11. Pozdnyakova O, Connell NT, Battinelli EM, Connors JM, Fell G, Kim AS. Clinical significance of CBC and WBC morphology in the diagnosis and clinical course of COVID-19 infection. *Am J Clin Pathol*. 2021;155(3):364–375. doi:10.1093/ajcp/aqaa231
12. Güçlü E, Kocayigit H, Okan HD, et al. Effect of COVID-19 on platelet count and its indices. *Revista da Associação Médica Brasileira*. 2020;66:1122–1127. doi:10.1590/1806-9282.66.8.1122
13. ElMaraghy AA, AbdelFattah EB, Ahmed MS. Platelet count: is it a possible marker for severity and outcome of community acquired pneumonia? *Egypt J Chest Dis Tuberc*. 2016;65(2):499–504. doi:10.1016/j.ejcdt.2015.09.001
14. Yin S, Huang M, Li D, Tang N. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *J Thromb Thrombolysis*. 2021;51(4):1107–1110. doi:10.1007/s11239-020-02105-8
15. Weinberg SE, Behdad A, Ji P. Atypical lymphocytes in peripheral blood of patients with COVID-19. *Br J Haematol*. 2020;190:24–39. doi:10.1111/bjh.16848
16. Fouad SH, Allam MF, Taha SI, et al. Comparison of hemoglobin level and neutrophil to lymphocyte ratio as prognostic markers in patients with COVID-19. *J Int Med Res*. 2021 Jul;49(7):03000605211030124
17. Kamel FO, Magadmi RM, Alqutub ST, et al. Clinical and hematologic presentations of adults with COVID-19 patients in Jeddah: a case control study. *J Infect Public Health*. 2021;14(6):709–716. doi:10.1016/j.jiph.2021.03.007
18. Paudel SS. A meta-analysis of 2019 novel Corona virus patient clinical characteristics and comorbidities; 2020.
19. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *bmj*. 2020;368

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Congenital Methemoglobinemia: First Confirmed Case in the Arab Population with a Novel Variant in the *CYB5R* Gene in the State of Qatar: A Case Report

Abdulrahman Al-Abdulmalek, Reem Al-Sulaiman, Mohammad Abu-Tineh & Mohamed A Yassin

To cite this article: Abdulrahman Al-Abdulmalek, Reem Al-Sulaiman, Mohammad Abu-Tineh & Mohamed A Yassin (2023) Congenital Methemoglobinemia: First Confirmed Case in the Arab Population with a Novel Variant in the *CYB5R* Gene in the State of Qatar: A Case Report, Journal of Blood Medicine, , 247-251, DOI: [10.2147/JBM.S395865](https://doi.org/10.2147/JBM.S395865)

To link to this article: <https://doi.org/10.2147/JBM.S395865>



© 2023 Al-Abdulmalek et al.



Published online: 31 Mar 2023.



Submit your article to this journal



Article views: 267



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Congenital Methemoglobinemia: First Confirmed Case in the Arab Population with a Novel Variant in the *CYB5R* Gene in the State of Qatar: A Case Report

Abdulrahman Al-Abdulmalek^{1,*}, Reem Al-Sulaiman^{2,*}, Mohammad Abu-Tineh^{2,*}, Mohamed A Yassin^{2,*}

¹Department of Internal Medicine, Hamad Medical Cooperation, Doha, Qatar; ²Department of Medical Oncology/Hematology, National Center for Cancer Care and Research, Doha, Qatar

*These authors contributed equally to this work

Correspondence: Mohammad Abu-Tineh, Department of Oncology/Hematology and BMT, National Center for Cancer Care and Research, Hamad Medical Corporation, Alrayan Road, Doha, Qatar, Tel +97455003969, Email Abutineh90@gmail.com

Abstract: Methemoglobinemia (MetHb) is a rare hematological condition characterized by high methemoglobin levels in the blood. It happens when hemoglobin is oxidized, resulting in hypoxia and cyanosis, which may occur in inherited or acquired forms. Inherited or congenital methemoglobinemia is a rare autosomal recessive condition and has never been reported in the Arab population. Here we report a case of a 22-year-old Arab man with a positive family history who presented with bluish discoloration of the fingers and lips and was found to have methemoglobinemia. Genetic study on the patient and his family revealed compound heterozygous variants in the *CYB5R* Exon 5 c.431G>A p.Gly144Asp likely pathogenic variant and *CYB5R* Exon 9 c.871G>A p.Val291Met variant of unknown significance. We suggest that the novel c.871G>A p.Val291Met variant could be causative for methemoglobinemia.

Keywords: methemoglobinemia, Arab, Qatar

Introduction

Methemoglobinemia (MetHb) is a rare hematological condition that occurs when hemoglobin is transformed into an oxidized form, resulting in a decrease in oxygen delivery to the tissues, causing hypoxia and cyanosis as common clinical manifestations.^{1,2} The symptoms' severity is directly proportional to the methemoglobin level. As it increases, the symptoms can progress, and the patient can develop headaches, fatigue, dizziness, acidosis, arrhythmia, seizures, and coma (MetHb >50%) which may lead to death (>70%).³ Methemoglobinemia can be acquired due to offending agents such as drugs or may be inherited or congenital. Methemoglobinemia is rare and less common than the acquired form and is caused by cytochrome *b*₅ reductase (*CYB5R*) deficiency and mutations in the *CYB5R* gene. The *CYB5R* gene encodes cytochrome *b*₅ reductase-3, an enzyme that catalyzes the transfer of reducing equivalents from NADH to cytochrome *b*₅. The soluble isoform of *CYB5R* is present in erythrocytes and significantly reduces methemoglobin to hemoglobin.² Biallelic variants in *CYB5R* have been reported in association with autosomal recessive congenital methemoglobinemia type I and type II. Type I methemoglobinemia is characterized by mild symptoms of cyanosis, whereas type II has more severe symptoms, including neurological manifestation.²

In this case report, we report the first case of congenital methemoglobinemia in the Arab population, specifically in the State of Qatar, with heterozygous compound variants in *CYB5R*, including a novel variant gene.

Case Presentation

A 22-year-old Arab Middle Eastern Qatari man with no significant past medical history presented to the hematology clinic to evaluate the bluish discoloration of his lips and extremities. The patient denied using any drugs, topical creams, or exposure to any exogenous agents. The patient also denied recent surgeries.

On physical examination, he was not in respiratory distress. Cyanosis was noticeable on the hands, toes, and lips (Figure 1A). Vital signs were stable and included an arterial oxygen saturation of 94–96% on room air. His blood tests showed hemoglobin (Hb) of 16.3, a mean corpuscular volume (MCV) of 88.8 fl, and normal kidney and liver function.

When the blood sample was obtained, it was noticed that the color of the blood was dark brown (Figure 1D). The arterial blood gas was obtained, resulting in a pH of 7.39, pCO₂ of 36.3 mmHg, and pO₂ of 121 mmHg.

Due to the apparent cyanosis that is not corrected with oxygen supplementation and normal pO₂, methemoglobinemia was suspected. Therefore, co-oximetry was performed on the same arterial blood sample, which showed a low FO₂Hb of 77.4 and a high methemoglobin level of 20.8%.

The patient reported a family history of a similar condition in his sister, who was also evaluated in the hematology clinic and was found to have methemoglobinemia. Given the patient's personal and family history, congenital methemoglobinemia was suspected. The patient and his sister were referred to the genetics clinic to rule out congenital methemoglobinemia in this family.

Family history revealed that the siblings are the product of consanguineous marriage (parents are first cousins), and no cases of methemoglobinemia or similar illnesses were reported in the family except for the two siblings, suggesting an autosomal recessive inheritance in this family given the consanguinity and the horizontal inheritance (Figure 2).

The Whole Exome Sequencing (WES) study was done on both siblings, including their mother. Both siblings carried heterozygous compound variants in the *CYB5R3* gene as follows:

First, *CYB5R3* Exon 5 c.431G > A p.Gly144Asp-likely pathogenic heterozygous variant (inherited from the mother). This variant was reported in the literature and was observed in a homozygous state in individuals with methemoglobinemia type I.⁴ Both the mother and the affected sibling are heterozygous for this variant.

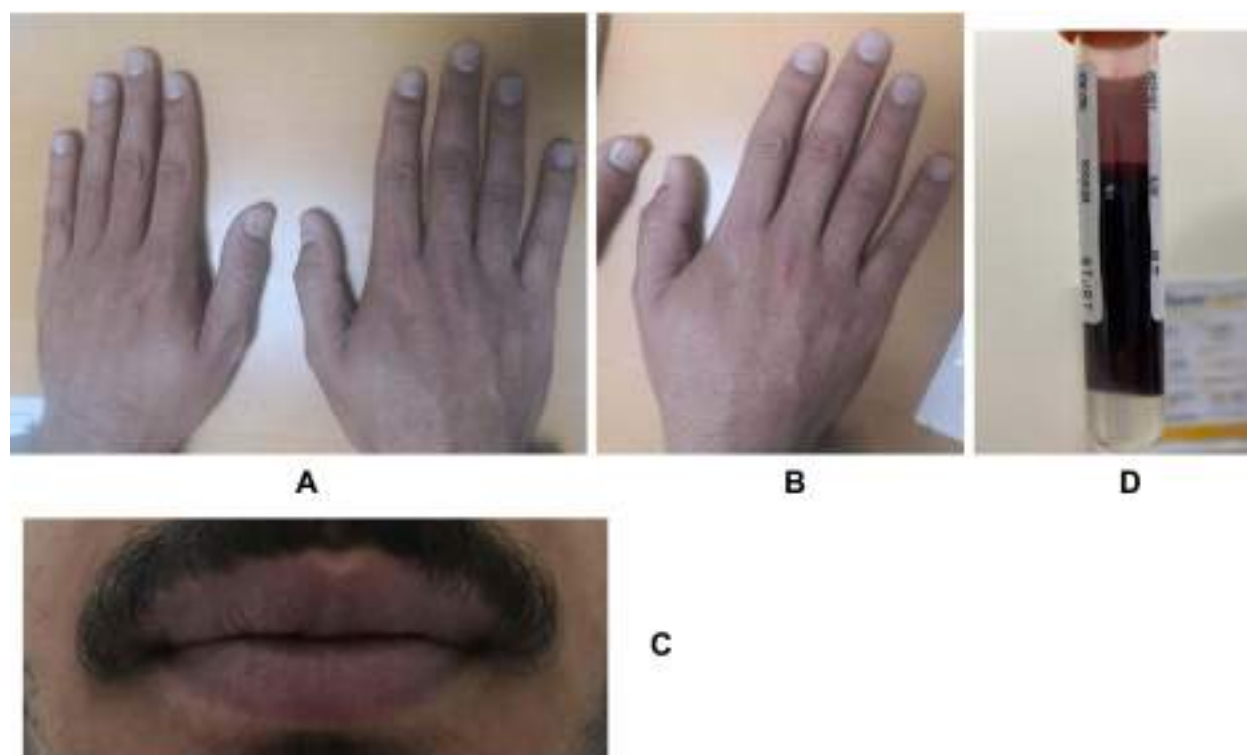


Figure 1 Showed cyanosis of the hands, fingers and lips(A–C). Also, it showed dark brown color blood (D). These findings are common manifestations of congenital methemoglobinemia.

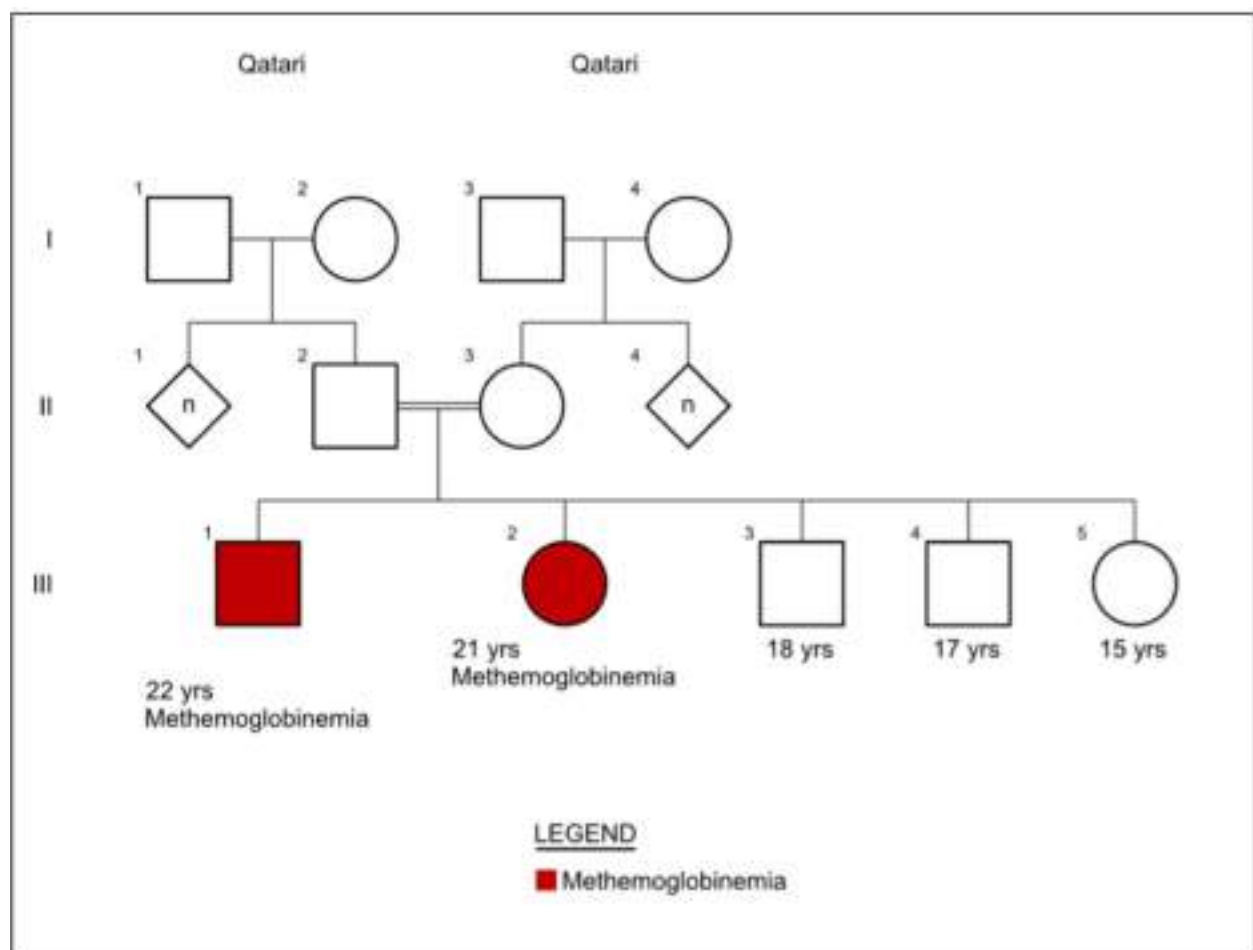


Figure 2 Autosomal recessive pedigrees tend to show fewer affected individuals and are often described as “skipping” generations. Thus, the primary feature distinguishing autosomal recessive from dominantly inherited traits is that unaffected individuals can have affected offspring, as seen by the red-colored affected children, from carrier parents from the second generation.

The second variant is CYB5R3 Exon 9 c.871G > A p.Val291Met-Variant of uncertain significance. This variant has not been previously published as pathogenic or benign. There are not enough data in large population cohorts to assess the frequency of this variant in publicly available databases.

Both siblings are carriers of this variant. However, the mother is not a carrier. This variant might have been inherited from the father. Neither the father nor the unaffected siblings could segregate this variant for its significance. Given that both affected siblings are carriers of this VUS, it is suggestive that this VUS might contribute to their phenotype and the likely pathogenic heterozygous variant.

A diagnosis of cytochrome *b*₅ reductase (CYB5R) deficiency was made, and the patient was treated with Vitamin C 500 mg daily. The patient showed significant improvement, evidenced by the reduced MetHb level and resolution of his cyanosis.

Discussion

Hemoglobin consists of four atoms of iron bound in a complex quaternary structure of proteins. In a normal hemoglobin molecule, iron is found in the ferrous state (Fe^{2+}), which facilitates the binding and delivery of oxygen. Methemoglobin is a condition wherein the iron exists in an oxidized state (Fe^{3+}) which has a reduced ability to bind to oxygen.^{4,5} Iron in the Fe^{3+} state affects the remaining ferrous ions (Fe^{2+}) to increase affinity for binding oxygen, resulting in impaired delivery of oxygen to the tissues. As a result, the oxygen–hemoglobin dissociation curve will be shifted to the left.^{5–7}

Methemoglobin forms as a result of oxidative stress and can form 0.5–3% per day, which is regulated by several counter-regulatory mechanisms to maintain a level of methemoglobin of less than 1%.⁴ A major pathway for the reduction of

methemoglobin back to hemoglobin is facilitated by the cytochrome *b*₅ reductase pathway, which is responsible for at least 95% of the methemoglobin reduction to hemoglobin. Another method is via the NAPH-methemoglobin reductase pathway, which exists as an alternative, accounting for 5%.⁷ Although the NADPH-reductase pathway is the responsible minor pathway, an exogenous pharmacological agent such as methylene blue can enhance it.^{2,5,8}

Two main ways may lead to increased methemoglobin levels. Firstly, a consequence of exposure to oxidizing agents, drugs, or metabolites causes the conversion of hemoglobin to methemoglobin to be increased by up to 1000-fold, overwhelming the regulatory mechanisms.¹ One oxidizing agent includes contaminated water, pharmaceutical drugs such as antimalarial drugs, dapsone, nitrites, and local anesthetic drugs such as lidocaine and benzocaine.^{1,3} Secondly, methemoglobin can be caused by genetic mutations that lead to intrinsic enzyme deficiencies in protective mechanisms, or the presence of abnormally structured hemoglobin M. These can be categorized into acquired and congenital (inherited) causes of methemoglobinemia, respectively.^{2,3}

This paper will focus on congenital causes. There are three kinds of hereditary methemoglobinemia, the most common being (CYB5R) deficiency. Less commonly reported are cytochrome *b*₅ deficiency and hemoglobin M disease. In hemoglobin M disease, an amino acid substitution mutation occurs, wherein tyrosine residues in the heme pocket replace histidine. In turn, heme-bound iron interacts with tyrosine phenolate sidechains and becomes resistant to reduction.^{10–12}

Cytochrome *b*₅ reductases are a group of essential enzymes present in all tissues; there are four known *CYB5R* genes that code for this protein, measuring approximately 32 kilobases in length and located on chromosome 22q 13-qter.² Mutations usually cause cytochrome *b*₅ reductase deficiency in one of the four *CYB5R* genes, which are usually inherited in an autosomal recessive fashion. Two types of CYB5R deficiency are categorized as type I and type II. In type I, the enzyme deficiency is restricted to erythrocytes; consequently, patients may present with asymptomatic cyanosis or mild symptoms.² Type II is much rarer, accounting for less than 10% of all cases of hereditary methemoglobinemia. This condition features a loss of CYB5R in all cells as opposed to type I. Patients generally have more severe manifestations of type II, such as neurological dysfunction and shortened life expectancy. Individuals with heterozygous variants in the *CYB5R3* gene are asymptomatic as they have some functional enzymes.^{2,6} However, they are at a higher risk of developing an acute presentation of clinically significant methemoglobinemia upon exposure to oxidizing agents.² However, phenotypical symptoms are usually present in individuals with homozygous variants.⁹

The distribution of the disorder is global; however, endemic zones exist. For example, the type I variant is reported more frequently in Athabascan Alaskans, Alaskan Eskimos,¹¹ Navajo Indians,^{13,14} Puerto Rico, and Yakutsk natives of Siberia.¹⁴

Cytochrome *b*₅ reductase deficiency is a rare disorder in the Arab population, with only one reported case in the literature.¹⁵ However, the case was diagnosed according to clinical presentation, measured methemoglobin levels, and cytochrome *b*₅ reductase enzyme activity. There have not been any reports in the literature of genetics-based diagnoses of this condition in the Arab population. Here, we are reporting the first case of type I Cytochrome *b*₅ reductase deficiency confirmed by genetic testing.

Conclusion

From the genetic study, it is suggested that the identified compound heterozygous variants in the *CYB5R3* gene might be causative of the congenital methemoglobinemia in our patients (most likely methemoglobinemia type I), supported by the clinical phenotype of the patients, the reported consanguinity in the family, and the autosomal recessive inheritance pattern. Future family segregation for the *CYB5R3* c.871G>A p.Val291Met variant (especially in newly affected cases) to better address its significance when it is inherited with the likely pathogenic variant c.431G>A p.Gly144Asp.

Abbreviations

MetHb, methemoglobinemia; CYB5R, cytochrome *b*₅ reductase; WES, whole exome sequencing; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate oxidase; Hb, hemoglobin; MCV, mean corpuscular volume.

Statement of Ethics

Written informed consent was obtained from our patient to allow the publication of information and images. Institutional approval was required for this case report. The case report was approved by the Hamad Medical Corporation's Medical Research Center – IRB – under the number MRC-04-23-043.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This article was funded by Qatar National Library. Open Access funding was provided by the Qatar National Library.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Cortazzo JA, Lichtman AD. Methemoglobinemia: a review and recommendations for management. *J Cardiothorac Vasc Anesth*. 2014;28(4):1043–1047. doi:10.1053/j.jvca.2013.02.005
2. Percy MJ, Lappin TR. Recessive congenital methaemoglobinemia: cytochrome b5 reductase deficiency. *Br J Haematol*. 2008;141(3):298–308. doi:10.1111/j.1365-2141.2008.07017.x
3. Camp NE. Methemoglobinemia. *J Emerg Nurs*. 2007;33(2):172–174. doi:10.1016/j.jen.2006.11.007
4. Kedar PS, Warang P, Nadkarni AH, Colah RB, Ghosh K A novel G143D mutation in the NADH-cytochrome b5 reductase gene in an Indian patient with type I recessive hereditary methemoglobinemia. *Blood Cells Mol Dis*. 2008;40(3):323–327. doi:10.1016/j.bcmd.2008.07.007
5. Percy MJ, Barnes C, Crighton G, et al. Methemoglobin reductase deficiency: novel mutation is associated with a disease phenotype of intermediate severity. *J Pediatr Hematol Oncol*. 2012;34(6):457–460. doi:10.1097/MPH.0b013e318257a492
6. Mansouri A, Lurie AA. Methemoglobinemia. *Am J Hematol*. 1993;42(1):7–12. doi:10.1002/ajh.2830420104
7. Neander NG, Loner CA, Rotoli JM. The acute treatment of methemoglobinemia in pregnancy. *J Emerg Med*. 2018;54(5):685–689. doi:10.1016/j.jemermed.2018.01.038
8. Skold A, Cosco DL, Klein R. Methemoglobinemia: pathogenesis, diagnosis, and management. *South Med J*. 2011;104(11):757–761. doi:10.1097/SMJ.0b013e318232139f
9. Kedar PS, Gupta V, Warang P, et al. Novel mutation (R192C) in CYB5R3 gene causing NADH-cytochrome b5 reductase deficiency in eight Indian patients associated with autosomal recessive congenital methemoglobinemia type-I. *Hematology*. 2018;23(8):567–573. doi:10.1080/10245332.2018.1444920
10. Carreira R, Palaré MJ, Prior AR, García P, Abrantes M. An unusual cause of neonatal cyanosis.... *Case Rep*. 2015;2015:bcr2014208371.
11. Alonso-Ojembarrena A, Lubián-López SP. Hemoglobin M disease as a cause of cyanosis in a newborn. *J Pediatr Hematol Oncol*. 2016;38(3):173–175. doi:10.1097/MPH.0000000000000489
12. Scott EM, Dale D. HOSKINS. “Hereditary methemoglobinemia in Alaskan Eskimos and Indians”. *Blood*. 1958;13(8):795–802. doi:10.1182/blood.V13.8.795.795
13. Balsamo P, Hardy WR, Scott EM. Hereditary methemoglobinemia due to diaphorase deficiency in Navajo Indians. *J Pediatr*. 1964;65(6):928–931. doi:10.1016/S0022-3476(64)80017-2
14. Scott RN, Ruiz-Bonilla JA, Christensen RD, et al. A patient with both methemoglobinemia and G6PD deficiency: a therapeutic conundrum. *Am J Hematol*. 2017;92(5):474. doi:10.1002/ajh.24683
15. Badawi MA, Badawi M, Wali S, et al. Hereditary methemoglobinemia manifesting in adolescence. *J Appl Hematol*. 2016;7(3):108. doi:10.4103/1658-5127.192983

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Prevalence of Inherited Thrombophilia in Women with Recurrent Pregnancy Loss During the First Trimester of Pregnancy

Tagwa Yousif Elsayed Yousif

To cite this article: Tagwa Yousif Elsayed Yousif (2023) Prevalence of Inherited Thrombophilia in Women with Recurrent Pregnancy Loss During the First Trimester of Pregnancy, Journal of Blood Medicine, , 253-259, DOI: [10.2147/JBM.S401469](https://doi.org/10.2147/JBM.S401469)

To link to this article: <https://doi.org/10.2147/JBM.S401469>



© 2023 Yousif.



Published online: 05 Apr 2023.



Submit your article to this journal



Article views: 366



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Prevalence of Inherited Thrombophilia in Women with Recurrent Pregnancy Loss During the First Trimester of Pregnancy

Tagwa Yousif Elsayed Yousif ^{1,2}

¹Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Jazan University, Gizan, 45142, Saudi Arabia; ²Department of Medical Laboratory Sciences, College of Medical Laboratory Sciences, Alneelien university, Khartoum, Sudan

Correspondence: Tagwa Yousif Elsayed Yousif, Email telsayed@jazanu.edu.sa

Purpose: This study aims to investigate the prevalence of inherited thrombophilia in women with recurrent pregnancy loss during the first trimester of pregnancy. The study was assessed the potential role of inherited thrombophilia in recurrent miscarriages and evaluate the consequences of this condition on the reproductive outcomes of affected women.

Material and Methods: This study was an analytical descriptive carried out in Khartoum, Sudan. The research comprised 98 controls who had given birth twice or more without experiencing a miscarriage and 120 patients. Each patient had done more than two miscarriages especially when the pregnancy is at its beginning trimester. (APCR), and (PS) were investigated using the clotting approach. There was an assessment of biological activities of (ATIII), (PC), and (PS) for both groups using the chromogenic method.

Results: The average age of the patients was 34, which was higher than the average age of the controls (33.5). The patient group had a much higher rate of multiple miscarriages among the women.: 35 (29.17%), 45 (37.50%), and 40 (33.33%). The incidence of PC deficiencies was determined to be 1.02% (1/98), whereas neither ATIII nor PS deficiencies were seen in the control group (0/98). APCR was more prevalent in the control group (4.10% or 4/98).

Conclusion: Despite contradicting evidence to the contrary in the literature, our findings imply that most miscarriages occur when pregnancy is at the first trimester when a woman is pregnant and they are all caused by thrombophilia.

Keywords: activated protein C resistance, antithrombin III, protein C, protein S, repeated miscarriages, thrombophilia

Introduction

Pregnancy loss that occurs three times in a row before the twentieth week of the last menstrual period is defined as recurrent pregnancy loss (RPL).^{1,2} Various underlying causes may be responsible for RPL, including uterine abnormalities, hormonal imbalances, autoimmunity, certain medications, chemicals, X-rays, and environmental factors. Additionally, genetic defects have also been identified as potential contributors. One such genetic defect is an alteration in the SERPINC1 (Serpin Family C Member 1) gene, which leads to an antithrombin III deficiency.^{3,4} Antithrombin III is a protein found in the blood and is responsible for keeping the balance between clotting and bleeding.⁵

Previous studies have indicated that thrombophilia, either inherited or acquired, is linked to recurrent pregnancy loss (RPL).⁶ Additionally, the relative risk of certain thrombophilia-related problems is often increased due to subpar case-control research designs, although the absolute risk is still extremely low.⁷ Antiphospholipid antibody syndrome should be thoroughly screened and undergo therapy if acquired thrombophilia is the primary cause of RPL.^{8,9}

Protein C and S are both glycoproteins produced by the liver and are essential for the body's natural anticoagulant system. Vitamin K, like its cofactor protein S, is also essential for protein C to function properly.¹⁰⁻¹² Activated protein C (APC) is produced when the coagulation factor thrombin is exposed to protein C. Protein C activity is within the normal range in a healthy, non-complicated pregnancy.¹³⁻¹⁵

Injection of heparin during pregnancy can prevent blood clots in women with antithrombin deficiency, although it is estimated that only 3% to 50% of pregnant women with this deficiency actually develop a clot.^{16,17} Despite the evidence being scant, anticoagulation is used to protect women with hereditary thrombophilia from having a negative pregnancy outcome.¹⁸ Additionally, the independent roles of protein S deficiency and miscarriage have rarely been studied.¹⁹

Despite the evidence linking thrombophilia to RPL, no systematic study has been performed to properly evaluate the role of genetic thrombophilia in RPL risk. Therefore, the aim of this study is to assess the role of genetic thrombophilia in recurrent pregnancy loss by conducting a comprehensive meta-analysis. By doing so, we hope to fill the existing gap in the knowledge and provide a clearer picture of the impact of genetic thrombophilia on pregnancy outcome.

Material and Methods

This study was an analytical descriptive carried out in Khartoum, Sudan, the Scientific Research Ethics Committee of Alneel University approved this research, which was done based on declaration of Helsinki. All participants in the study gave their informed consent. 120 women who had experienced two or more miscarriages in the first trimester of their pregnancies made up the study group (patient group), as contrasted to 98 women in the control group who had delivered two or more infants without experiencing a miscarriage. Abortions in the first trimester of pregnancy are common, and the investigation could not rule out the possibility that these abortions were caused by chromosomal abnormalities, infections, anatomical abnormalities, or endocrine dysfunction. Women who were pregnant at the time of the study were barred from taking part in either study.

The plasma was separated by mixing 9 parts blood with 1 part sodium citrate (3.2%) and spinning the mixture at 2000 revolutions per minute (rpm) for 15 minutes. After collecting the plasma, it was kept at 35 degrees Celsius until testing could be performed. For the purpose of a hemostasis test. Chromogenic measurements of ATIII and PC were taken with the help of an automated coagulometer and the TEChrom AT (anti-Xa) and TEChrom PC kits from TECO GmbH. (Coatron 6). PS and APCR were evaluated utilizing the Coatron 6 and Amelung KC4 semi-automatic coagulometers, amongst other methods.

The odds ratio (OR) and 95% confidence interval were determined for each inhibitor separately. The Chi-square test in IBM SPSS V.22 was then used to assess APCR. We used a significance level of P 0.05 for all *t*-test comparisons.

Findings

One hundred and twenty women who had experienced two or more miscarriages during the first trimester of pregnancy (the patient group) and ninety-eight women (the control group) who had experienced two or more normal deliveries (without a miscarriage) were tested for the natural coagulation inhibitors ATIII, PC, PS, and APCR.

The frequency of errors in ATIII, PC, and PS are provided together with APCR. APCR, ATIII, PC, and PS deficits were seen in the patient group at rates of 5.00% (6/120), 4.17% (5/120), 5.00% (6/120), and 7.50% (9/120), respectively. While there were no ATIII or PS deficits in the control group, there were 1% (1.02%) (1/98) PC inadequacies. APCR was more prevalent in the control group (4.10% or 4/98). The ORs are 4.08 (0.49–34.4), 1.84 (0.585–7.9), and 4.25 (1.57–11.47), respectively, for PC, APCR, and total problems (Table 1).

Table 1 The Prevalence of Antithrombin III, Protein C and Protein S Deficiencies and the Prevalence of APCR in the Women with RLP and in the Controls

	Patients 120 N (%)	Controls 98 N (%)	Odds Ratio (95% CI)	P
AT III	6/120 (5.00)	0/98 (0)	4.08 (0.49–34.4)	NS
PC	5/120 (4.17)	1/98 (1.02)		
PS	6/120 (5.00)	0/98 (0)		
APCR	9/120 (7.50)	4/98 (4.10)	1.84 (0.58–5.79)	NS
Total	26/120 (21.67)	5/98 (5.10)	4.25 (1.57–11.47)	NS

Abbreviations: N, Number; NS, non-significant.

The data demonstrated no statistically significant difference in mean impairment scores between patients and controls on the ATIII, PC, PS, or APCR. The prevalence of ATIII, PC, and PS deficits, as well as APCR and Totals, did not differ significantly between the groups (Table 1).

In both controls and RLP-positive women, there were impairments in the prevalence of protein C, protein S, antithrombin III, and APCR. The distribution and average value of the frequency with which each group has ATIII, PC, and PS impairments. Discovered that ATIII, PC, and PS impairments in both populations result from heterozygote abnormalities. Patients had APCR-SR values between 1.45 and 2.10, while controls had values between 1.46 and 2.20, demonstrating the same trend (Table 2). With the methods were used, the normal range for ATIII, PC, and PS is 70–135%; however, the normal range for APCR-SR is greater than 2.2 (>2.2).

The median age of patients was 34 (range: 21–45), while it was 33 in the comparison group. The patients were 34.3 years old on average (SD 5.72). (SD 5.80) (Table 3).

In the patient group, 35 (29.17%) women had a history of 3 miscarriages, 45 (37.50%) had a history of 4 miscarriages and 40 (33.33%) had a history of more than 4 miscarriages (Table 4).

Correlations

For exploring of the relationships between different variables. The hypothesis of association will be significant if $P < 0.05$. Ran tests of association for various variables and the results were as follows. $P = 0.000$ which is highly significant. It is

Table 2 The Distribution and Mean Values of ATIII, PC, PS, and APCR Deficiency Frequencies, as Well as Their Frequencies Within Each Group, Were Calculated

	Patients			Controls		
	N	Range	Mean Value	N	Range	Mean Value
AT III	6	46–59	59.0	—	—	—
PC	5	45–52	47.2	1	—	48
PS	6	44–66	52.0	—	—	—
			Mean APCR-SR			Mean APCR-SR
APCR	9	1.45–2.10	1.70	4	1.46–2.20	1.78

Abbreviations: N, Number; APCR-SR, Activated protein C – Sensitivity Ratio.

Table 3 Summary of the Age of the Two Groups

	Count	Minimum	Maximum	Mean	Median	Standard Deviation
Patient	120	21.0	45.0	34.3	34.0	5.7
Control	98	21.0	45.0	33.5	34.0	5.8

Table 4 Frequencies of the History of Miscarriages in Both Groups

		CASE			
		Patient		Control	
		Count	Column N %	Count	Column N %
No.of Miscarriages	0.0	0	0.0%	98	100.0%
	3.0	35	29.2%	0	0.0%
	4.0	45	37.5%	0	0.0%
	5.0	31	25.8%	0	0.0%
	6.0	9	7.5%	0	0.0%

evident that the association between age and number of miscarriages for the patient group is significant. This suggests that the frequency of miscarriages among patients is significantly influenced by their age (Table 5).

The results show there is no evidence that age of the patients affect the levels of ATIII, PC, PS or APCR to a significant level. It is not clear from the data above that the control group's older age significantly influences the levels of ATIII, PC, PS, or APCR (Table 6).

The foregoing data do not clearly show that ATIII, PC, PS, or APCR have a substantial impact on miscarriage rates. The connections between the various protein level pairings in the patients, however, are noteworthy (Table 7).

Table 5 Relationship Between Age and Number of Miscarriages for the Patients

Correlation Between Age and No. of Miscarriages	
Pearson Correlation	0.457**
Sig. (2-tailed)	0.000
N ^a	120

Notes: **Correlation is significant at the 0.01 level (2- tailed). ^aCASE = Patient.

Table 6 Relationship Between Age and ATIII, PC, PS and APCR in Patients' and Control Groups

Correlations					
Patients		AT.III	PC	PS	APCR
Age	Pearson Correlation	−0.046	−0.004	−0.045	−0.187
	Sig. (2-tailed)	0.618	0.964	0.623	0.041
	N	120	120	120	120
Controls		AT.III	PC	PS	APCR
Age	Pearson Correlation	−0.213	−0.052	0.175	0.002
	Sig. (2-tailed)	0.035	0.611	0.084	0.981
	N	98	98	98	98

Notes: Correlation is significant at the 0.05 level (2-tailed). Correlation is significant at the 0.01 level (2-tailed).

Table 7 A Correlation Between the Number of Miscarriages, ATIII, PC, PS, and APCR in the Patient Group's

Correlations ^a		AT.III	PC	PS	APCR
Number of miscarriages	Pearson Correlation	0.020	−0.032	−0.045	−0.083
	Sig. (2-tailed)	0.830	0.730	0.627	0.368
	N	120	120	120	120
AT.III	Pearson Correlation		0.188	0.328	0.199
	Sig. (2-tailed)		0.039	0.000	0.029
	N		120	120	120
PC	Pearson Correlation			0.296	0.288
	Sig. (2-tailed)			0.001	0.001
	N			120	120
PS	Pearson Correlation				0.264
	Sig. (2-tailed)				0.004
	N				120

Notes: Correlation is significant at the 0.05 level (2-tailed). Correlation is significant at the 0.01 level (2-tailed). ^aCASE = Patient.

Table 8 Relationship Between the Protein Level and the Group (Patient or Control) (Normal or Deficient)

Chi-squares Between Group and the Different Protein Levels			
	Protein Level	Chi-Square Value	Sig. (2-Tailed)
Group	ATIII	5.039	0.025
	PC	1.995	0.158
	PS	5.039	0.025
	APCR	1.124	0.289

Table 9 T-Test for ATIII, PC, PS and APCR Protein Levels That are Group-Independent Samples

		t-test for Equality of Means						
		t	df	Sig. (2-Tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
AT.III	Equal variances assumed	1.175	216	0.241	2.6665	2.2685	-1.8047	7.1377
	Equal variances not assumed	1.202	215.919	0.231	2.6665	2.2186	-1.7064	7.0394
PC	Equal variances assumed	2.801	216	0.006	6.2257	2.2228	1.8444	10.6069
	Equal variances not assumed	2.935	200.713	0.004	6.2257	2.1215	2.0423	10.4090
PS	Equal variances assumed	1.826	216	0.069	4.2993	2.3542	-0.3408	8.9394
	Equal variances not assumed	1.883	213.482	0.061	4.2993	2.2826	-0.2001	8.7987
APCR	Equal variances assumed	-3.566	216	0.000	-0.24877	0.06976	-0.38627	-0.11128
	Equal variances not assumed	-3.467	176.633	0.001	-0.24877	0.07175	-0.39037	-0.10718

Employing the Chi-square test, can determine if there is a statistically significant connection between the patient and control groups with regards to their protein status. The table below displays the outcomes. According to the data above, ATIII and PS are considerably related with the group of women, however PC and APCR are not significantly connected with the women's group (Table 8).

These findings show that the means for PC and APCR for the two groups of women are considerably different, although the results for ATIII and PS (for which we have already established that they are not independent in the previous sections) are inconsequential (Table 9).

Discussion

This study aimed to determine if and how activation protein C resistance (APCR), protein C and protein S deficiency, and antithrombin III (ATIII) levels were associated with repeated losses in the first trimester of pregnancy. One hundred twenty individuals with recurrent pregnancy loss (RPL) and ninety-eight healthy controls underwent tests for ATIII, PC, and PS deficiencies, as well as the existence of APCR. According to the current study, deficiencies in APCR, AT, PC, and PS may be found in both healthy individuals and those who have experienced thromboembolic events. Although it is yet unknown whether or not genetic thrombophilia plays a role in recurrent miscarriages, more patients than controls had ATIII, PC, or PS deficits, and more patients with APCR (2.99 for the minimum, 4.50 for the maximum, and 2.99 for the mean, compared to 2.23, 3.90, and 2.77 for the patient group).

However, ethical considerations should be taken into account when conducting studies of this nature.²⁰ Women should be made aware of the potential risks associated with testing for genetic thrombophilias, such as false positives or

inaccurate results. In addition, it is important that women are not pushed into testing if they do not feel comfortable doing so. Furthermore, informed consent must be obtained from all participants and any results should be kept confidential.

Overall, it is clear that there's a need for better study designs to determine if a lack of ATIII, PC, or PS, or the presence of any other factor, was responsible for the patients' recurrent miscarriages. Because of this, Walker et al propose that women who have them undergo frequent prenatal testing.^{12,21,22} However, hereditary thrombophilia testing is unnecessary for women experiencing repeated miscarriages or problems with late pregnancy outside of clinical research, as revealed by Patnaik et al^{11,12,23} Therefore, if a woman is at a higher risk of developing venous thromboembolism during pregnancy, she should be advised to use graduated compression stockings as early as possible in her pregnancy. Vascular uteroplacental insufficiency has been linked to an increased risk of venous thromboembolism, as well as acquired or hereditary thrombophilia.^{13,23}

Conclusion

Research found that there were associations between different pairs of protein levels in patient groups, suggesting that thrombophilia may have a role in first-trimester losses.

Disclosure

The author reports no conflicts of interest in this work.

References

- Kupfermine MJ. Thrombophilia and pregnancy. *Reprod. Biol. Endocrinol.* **2003**;1(1):111. doi:10.1186/1477-7827-1-111
- Brill-Edwards P, Ginsberg JS, Gent M. Safety of withholding antepartum heparin in women with a previous episode of venous thromboembolism. *N Engl J Med.* **2000**;343(20):1439–1444. doi:10.1056/nejm200011163432002
- Martinelli I. Thromboembolism in women. *Semin Thromb Hemost.* **2006**;32(7):709–715. doi:10.1055/s-2006-951455
- Robertson L, Wu O, Langhorne P, et al. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: risk and Economic Assessment of Thrombophilia Screening (TREATS) study. *Br J Haematol.* **2005**;132(11):171–196. doi:10.3310/hta10110
- Simcox LE, Ormesher L, Tower C, Greer IA. Thrombophilia and Pregnancy Complications. *Int J Mol Sci.* **2015**;16(12):28418–28428. doi:10.3390/ijms161226104
- Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol.* **1994**;87(1):106–112. doi:10.1111/j.1365-2141.1994.tb04878.x
- Gerhardt A, Scharf RE, Beckman MW. Prothrombin and factor V mutations in women with thrombosis during pregnancy and the puerperium. *N Engl J Med.* **2000**;342(6):374–380. doi:10.1056/NEJM200002103420602
- Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: a systematic review. *Br J Haematol.* **2006**;132(2):171–196. doi:10.1111/j.1365-2141.2005.05847.x
- Liu X, Chen Y, Ye C, et al. Hereditary thrombophilia and recurrent pregnancy loss: a systematic review and meta-analysis. *Int J Mol Sci.* **2021**;36(5):1213–1229. doi:10.1093/humrep/deab010
- Kluijtmans LA, van den Heuvel LP, Boers H, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet.* **1996**;58(1):35–41. PMID: 8554066.
- Patnaik MM, Haddad T, Morton CT. Pregnancy and thrombophilia. *Semin Respir Crit Care Med.* **2007**;28(5):753–765. doi:10.1586/14779072.5.4.753
- Walker ID. Thrombophilia in pregnancy. *J Clin Pathol.* **2000**;53(8):573–580. PMID: 11002758. doi:10.1136/jcp.53.8.573
- Bates SM. Management of pregnant women with thrombophilia or a history of venous thromboembolism. *Hematology Am Soc Hematol Educ Program.* **2007**;1(1):143–150. doi:10.1182/asheducation-2007.1.143
- McNamee K, Dawood F, Farquharson RG. Thrombophilia and early pregnancy loss. *Best Pract Res Clin Obstet Gynaecol.* **2012**;26(1):91–102. doi:10.1016/j.bpobgyn.2011.10.002
- Arachchillage DJ, Mackillop L, Chandratheva A, Motawani J, MacCallum P, Laffan M. Thrombophilia testing: a British society for haematology guideline. *Br J Haematol.* **2022**;198(3):443–458. doi:10.1111/bjh.18239
- Mekaj Y, Lulaj S, Daci F, et al. Prevalence and role of antithrombin III, protein C and protein S deficiencies and activated protein C resistance in Kosovo women with recurrent pregnancy loss during the first trimester of pregnancy. *J Hum Reprod Sci.* **2015**;8(4):224–229. PMID: 26752858. doi:10.4103/0974-1208.170407
- Preston FE, Rosendaal FR, Walker ID, et al. Increased fetal loss in women with heritable thrombophilia. *Lancet.* **1996**;348(9032):913–916. doi:10.1016/s0140-6736(96)04125-6
- Alecsandru D, Klimczak AM, Velasco AG, Pirtea P, Franasia JM. Immunologic causes and thrombophilia in recurrent pregnancy loss. *Fertil Steril.* **2021**;115(3):561–566. doi:10.1016/j.fertnstert.2021.01.011
- Dizon-Townson D, Miller C, Sibai B, et al. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol.* **2005**;106(3):517–524. doi:10.1097/01.AOG.0000173986.32528.ca
- Hatasaka HH. Recurrent miscarriage: epidemiologic factors, definitions, and incidence. *Clin Obstet Gynecol.* **1994**;37(3):625–634. doi:10.1097/00003081-199409000-00016

21. Scharrer I, Hach-Wunderle V, Aygoren E, et al. Frequency and clinical manifestations of congenital plasminogen and fibrinogen deficiency compared to AT III-, PC-, and PS- deficiency in patients suffering from thromboses. *Fibrin Proteolysis*. 1988;2(2):16–17.
22. Barbour LA, Oja JL, Schultz LK. A prospective trial that demonstrates that dalteparin requirements increase in pregnancy to maintain therapeutic levels of anticoagulation. *Am J Obstet Gynecol*. 2004;191(3):1024–1029. doi:10.1016/j.ajog.2004.05.050
23. GriYn JH, GriYn JH, GriYn JH, et al. Anticoagulant protein C pathway defective in a majority of thrombophilic patients. *Blood*. 1993;82(7):1989–1993. doi:10.1182/blood.V82.7.1989.1989

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Chronic Myeloid Leukemia, from Pathophysiology to Treatment-Free Remission: A Narrative Literature Review

Ikhwan Rinaldi & Kevin Winston

To cite this article: Ikhwan Rinaldi & Kevin Winston (2023) Chronic Myeloid Leukemia, from Pathophysiology to Treatment-Free Remission: A Narrative Literature Review, Journal of Blood Medicine, , 261-277, DOI: [10.2147/JBM.S382090](https://doi.org/10.2147/JBM.S382090)

To link to this article: <https://doi.org/10.2147/JBM.S382090>



© 2023 Rinaldi and Winston.



Published online: 06 Apr 2023.



Submit your article to this journal



Article views: 2054



View related articles



View Crossmark data



Citing articles: 17 View citing articles

Chronic Myeloid Leukemia, from Pathophysiology to Treatment-Free Remission: A Narrative Literature Review

Ikhwan Rinaldi ¹, Kevin Winston ^{2,3}

¹Division of Hematology and Medical Oncology, Department of Internal Medicine, Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; ²Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; ³Hospital Medicine, Bhakti Medicare Hospital, Sukabumi, Indonesia

Correspondence: Ikhwan Rinaldi, Division of Hematology and Medical Oncology, Department of Internal Medicine, Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, Email ikhwanrinaldi@gmail.com

Abstract: Chronic myeloid leukemia (CML) is one of the most common leukemias occurring in the adult population. The course of CML is divided into three phases: the chronic phase, the acceleration phase, and the blast phase. Pathophysiology of CML revolves around Philadelphia chromosome that constitutively activate tyrosine kinase through BCR-ABL1 oncoprotein. In the era of tyrosine kinase inhibitors (TKIs), CML patients now have a similar life expectancy to people without CML, and it is now very rare for CML patients to progress to the blast phase. Only a small proportion of CML patients have resistance to TKI, caused by BCR-ABL1 point mutations. CML patients with TKI resistance should be treated with second or third generation TKI, depending on the BCR-ABL1 mutation. Recently, many studies have shown that it is possible for CML patients who achieve a long-term deep molecular response to stop TKIs treatment and maintain remission. This review aimed to provide an overview of CML, including its pathophysiology, clinical manifestations, the role of stem cells, CML treatments, and treatment-free remission.

Keywords: chronic myeloid leukemia, remission, tyrosine kinase inhibitor

Introduction

Chronic myeloid leukemia or chronic myelogenous leukemia (CML) is a hematopoietic stem cell (HSC) disorder, as are all leukemias. In CML, the disorder is characterized by translocation t(9;22)(q34;q11), resulting in the fusion of BCR and ABL1 genes into the pathogenic BCR-ABL1 oncogene, with many subsequent effects on downstream pathways.^{1,2} The main pathway effect of this fused oncogene is to activate tyrosine kinase pathway constitutively, resulting in a proliferative advantage of the mutant HSCs compared to normal HSCs, and the gradual displacement of normal HSCs.³

CML symptoms range from asymptomatic to overt leukostasis, depending on the stage of CML; leukostasis often occurs in the blast phase. Meanwhile, hyperleukocytosis is the most common symptom, and is often present in all phases (chronic phase, accelerated phase, and blast phase).¹ The blast phase is similar to acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). The diagnosis of CML often requires a basic hematology workup combined with identification of chromosomal abnormalities. Different phases of CML can be differentiated by clinical symptoms and blast percentage.

CML is one of the model diseases for targeted therapy. It is widely accepted that discovery of tyrosine kinase inhibitors (TKIs) has changed the treatment landscape of CML.⁴ Prior to the introduction of TKIs, CML was universally fatal in the long term. However, since TKIs have become the first-line treatment for CML, the life expectancy of CML patients has become similar to that of people without CML.^{5,6}

While TKIs have indeed improved the survival of CML patients, there are still unresolved issues associated with TKI treatment, such as adverse side effects and impact on quality of life. Furthermore, a very important issue associated with TKI treatment is the need for most patients to continuously take the TKI to prevent relapse. Thus, many patients are on

lifelong TKI treatment. However, several studies, such as STIM1 trial, have shown that patients with a deep molecular response (DMR) can achieve treatment-free remission (TFR) with no relapse after discontinuing their TKI.⁷ This review article aimed to discuss all aspects of CML.

Pathophysiology of CML

The presence of BCR-ABL1 fusion gene in hematopoietic stem cells has been shown to be sufficient for initiation of CML.⁸ Thus, the pathophysiology of CML revolves around the BCR-ABL1 fusion gene. Approximately 90% to 95% of patients with CML have reciprocal translocation of t(9;22) (q34;q11.2).^{1,2} This results in a shortened chromosome 22, termed Philadelphia chromosome, containing the BCR-ABL1 oncogene. However, It should be noted that BCR-ABL1 may also be present in other types of leukemia, such as acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).⁹ The significance of BCR-ABL1 in ALL and AML is still under investigation.

At the time of writing, the mechanism of Philadelphia chromosome formation and the time from the formation of the chromosome to the occurrence of clinical symptoms of CML are still not known with certainty. The Philadelphia chromosome was previously thought to have been formed due to the influence of radiation from the atomic bombs of Hiroshima and Nagasaki on CML patients in Japan.¹⁰ Indeed, in vitro studies have shown that exposure of myeloid cell lines to high-dose radiation causes the expression of BCR-ABL1.¹⁰ However, the majority of people are not exposed to chronic radiation. Some experts believe that the Ph chromosome can be formed as a result of spontaneous random translocation. Supporting this is the fact that the BCR-ABL1 gene has also been observed in a small proportion of healthy individuals.^{11,12} This could also mean that not all BCR-ABL1 fusion genes develop into CML. Further studies are needed to elucidate the origins of the BCR-ABL1 gene; however, it will be very difficult to isolate the exact causes that contribute to the formation of the Philadelphia chromosome due to the relative rareness of the disease and issues with determining the onset of the disease, as it is initially asymptomatic.

As has been described by much of the scientific literature on this topic, the BCR-ABL1 hybrid gene located on the Ph chromosome synthesizes the oncoprotein 210 kD (p210), which plays a role in leukemogenesis of CML.¹³ The p210^{BCR-ABL1} protein can continuously activate the tyrosine kinase pathway, resulting in an increase in the signal transduction of unregulated downstream oncogenic pathways such as JAK/STAT, PI3K/AKT, RAF, MYC, and RAS/MEK.^{3,13} All of these pathways are involved in cell growth, cell survival, and the inhibition of apoptosis. Thus, HSCs with BCR-ABL1 translocation have a proliferative advantage due to their enhanced response to growth factors. Furthermore, proliferation-inhibitory factors are less responsive. Thus, overall, the presence of BCR-ABL1 in hematopoietic stem cells will encourage their transformation into leukemia stem cells (LSCs) and replacement or displacement of normal hematopoietic stem cells.

In CML, there are other types of BCR-ABL1 fusion and breakpoints. For example, p190^{BCR-ABL1} (e1a2), which encodes a hybrid 190 kDa protein, can be observed in CML, but the frequency is rare. Based on several studies, p190^{BCR-ABL1} occurs in only 1–2% of CML patients.^{14,15} Other BCR-ABL1 proteins include p230^{BCR-ABL1}.

A point of interest is that not all CML patients have Philadelphia chromosome. In a minority of CML patients, the Philadelphia chromosome is lacking but identifiable BCR-ABL1 oncogenes are present. Observations have shown that the number of confirmed CML patients with no Philadelphia chromosome is up to 5–10%.^{16–18} Interestingly, approximately 25–50% of these patients still have BCR-ABL1 gene rearrangement, as confirmed by molecular examinations.^{18,19} In other words, the BCR-ABL1 genes in this subset of CML patients originate outside of the Philadelphia chromosome. All other patients have no confirmed BCR-ABL gene rearrangement.

According to a study by Wang et al, BCR-ABL-negative CML patients have a worse overall survival and have a higher risk of their leukemia transforming into acute myeloid leukemia.²⁰ Without the presence of BCR-ABL, most TKIs are not indicated as a main treatment, which may be one of the factors contributing to a worse prognosis in BCR-ABL-negative CML patients. Gene mutations commonly found in BCR-ABL-negative CML include SETBP1, ASXL1, NRAS/KRAS, SRSF2, CSF3R, U2AF1, and many others.²¹ Some of these mutations may be used as potential targets for therapy in BCR-ABL-negative CML, but the evidence to support this is currently lacking.

Pathophysiology of CML Progression

CML is grouped into three phases, namely the chronic phase, the accelerated phase, and the blast phase.¹ In the chronic phase, LSCs are confined to hematopoietic tissues. However, in the blast phase, the LSCs gain an invasive capability that enables them to infiltrate other organs, most notably the spleen, resulting in splenomegaly. Furthermore, immature blast cells now dominate in blast phase. The process of transformation from chronic CML to the accelerated phase or blast phase is thought to be caused by amplification of the BCR-ABL1 protein and increased activation of the downstream tyrosine kinase pathway, combined with the emergence of additional chromosomal abnormalities (ACAs).^{22,23} Oxidative stress and impaired DNA repair have also been implicated.^{24–26} In addition, mutations such as deletion of the IKZF1 gene and loss of CCAAT protein expression have been reported to cause the immaturity of granulocytes.^{27,28} Blast-phase patients often have multiple gene mutations.²⁹ Common gene mutations due to genomic instability in blast-phase CML are summarized in Table 1.^{22,30,31}

Additional Chromosomal Abnormalities in CML

LSCs are genetically unstable, so they have a tendency to present with other genomic or cytogenetic abnormalities.^{32,33} Additional cytogenetic abnormalities (ACAs) have been shown to increase in the later phases of CML. For example, in the chronic phase or at diagnosis, only approximately 5–10% of CML patients have ACAs, but this increases to up to 80% in the blast phase.^{34,35} Cytogenetic changes commonly observed in the blast phase include +8, +19, double Philadelphia chromosome, and isochromosome 17q.^{36,37}

The presence of ACAs in the early stages appears to be associated with disease progression in CML. A study by Clark et al showed poor progression-free survival in CML patients with ACAs.³⁷ Additionally, a study by Chandran et al also found a poor prognostic effect of ACAs in CML.³⁵ The latest CML guidelines from the European LeukemiaNet state that the appearance of ACAs is one of the early indicators of disease progression, and the high-risk type of ACAs are associated with TKI treatment resistance.³⁸ A study by Meggyesi et al showed that 30 out of 65 imatinib-resistant patients (46%) had ACAs.³⁹ Monitoring of ACAs during TKI treatment is important as ACAs are associated with treatment resistance.⁴⁰

CML Stem Cells

Evidence of the HSC origin of CML comes from the observation that the translocation of BCR-ABL1 is observed in myeloid, erythroid, and megakaryocytic cells. However, the effects of this translocation on erythroid and megakaryocytic cells are still unclear. The suppression of erythroid and megakaryocytic cells in CML is very likely due to the high proliferation of myeloid cells causing disruption. The malignant HSCs, termed leukemia stem cells (LSCs), provide a reservoir of self-replenishing leukemia cells.² Thus, to completely eradicate CML, LSCs ideally must be targeted and removed to prevent relapse.

Table 1 Common Gene Mutations in Blast-Phase CML

Gene Mutations	Gene Functions
p53 loss of function	Growth arrest, DNA repair, and apoptosis
CDKN2A loss of function	Tumor suppressors
GATA-2 gain of function	Gene expression regulation through transcription factor
RUNX1 mutations	Hematopoietic stem cell maturation
IKZF1 mutations	Regulator of lymphoid differentiation
ASXL1 mutations	Gene transcription regulator
WT1 mutations	Tumor suppressor

LSCs in chronic-phase CML can be identified via positive CD34 markers and negative CD38 markers.^{2,41–43} However, using only these surface antigen markers provides a heterogeneous population, and these markers are not unique to CML LSCs.⁴¹ For example, the population of normal HSCs can also be identified using positive CD34 markers and negative CD38 markers as well. CD33 marker is also expressed by both normal stem cells and LSCs. However, there is a greater density of CD33 in LSCs.⁴⁴ Indeed, LSC identification is also a challenge in other cancers.⁴⁵

In an article by Hoshmand et al, it is stated that markers such as CD25, CD26, and interleukin-1-receptor accessory protein (IL1RAP) have been reported to be expressed specifically on CML stem cells.⁴¹ CD25 is encoded by the IL2RA gene and the CD25 marker has been reported to be expressed by more than 90% of CD34⁺/CD38[−] LSCs.^{46,47} Meanwhile, neither CD26 nor IL-1RAP have been shown to be expressed in LSCs in most other malignancies.⁴⁸ However, it should be noted that both CD26 and IL-1RAP are expressed in normal mast cells, which may complicate identification.^{49,50} There are some possible combinations of cell surface markers for reliable identification of CML LSCs such as Lin-negative, CD34-positive, CD38-negative/low, CD45RA-negative, KIT-negative, and CD26-positive but this requires further confirmations.^{41,51}

LSCs have a persistent presence, even with tyrosine kinase inhibitor treatment.⁵² Thus, patients in remission are not “cured” and may technically relapse once TKI treatment is stopped. This suggests that there may be BCR-ABL1-independent pathways that maintain the self-renewal of CML LSCs. A study by Zhao et al showed that the maintenance of cancer stem cells requires activation of Hedgehog signaling.⁵³ Additionally, a study by Chen et al demonstrated that the Alox5 gene is important for LSC survival.⁵⁴ Other pathways or processes that support CML LSCs persistence include JAK1-STAT3 signaling, PI3K/AKT/FOXO signaling, Wnt signaling, and impaired DNA damage responses.^{55–59} LSCs also have increased expression of IL-1 receptors, which, if activated, reduce the growth of LSCs.⁶⁰ Other cytokines that provide support to LSCs include TNF-alpha and IL-6.⁶¹ Thus, it is not unexpected that a significant proportion of patients in remission relapse after stopping treatment.

Therapies to target CML LSCs has not been used clinically. One may argue that since long-term sustained molecular responses from TKI produce a significant proportion of patients remaining in molecular response when stopping TKI, therapies to target CML LSCs may not be needed. However, there will always be a relatively high proportion of patients that will not be able to achieve TFR status and thus, therapies targeting CML LSCs may be useful for this population. We the authors speculate that using combination therapies of TKI with one or several drugs targeting LSCs pathways may be the new norm in the future. EZH2 inhibitor and JAK inhibitor combine with TKI seem to be effective in eliminating LSCs and should be further researched.^{62,63}

Clinical Manifestations and Diagnosis

As stated above, the majority of CML patients are asymptomatic, especially in the early stages.¹ Patients are often suspected to have CML following a medical check-up revealing leukocytosis and basophilia. In the chronic phase, when the majority of CML patients are diagnosed, the symptoms, if there are any, are fatigue, weight loss, cold sweats, and abdominal pain or feeling full quickly due to splenomegaly. Spleen size should be measured, as it is a component of CML prognosis scores such as the Sokal score and the ELTS score. Other symptoms that can occur, albeit rarely, are low-grade fever, bleeding, thrombosis, gouty arthritis, priapism, and gastric ulcers. The baseline diagnostic workup recommended by the ELN 2020, ESMO 2017, and NCCN 2021 guidelines is presented in Table 2.^{38,64,65}

Upon routine hematological examination, an increase in the number of leukocytes and a shift in the differential count to the left, and myeloid series cells in various stages of differentiations, are often found.⁶⁶ Leukocyte counts are often above $25 \times 10^9/L$ with an increase in basophil count.⁶⁷ In the chronic phase, the blast percentage is often low or negative. Platelets are also elevated in the majority of patients.

Bone marrow examination reveals a hypercellular marrow with more than 75% cellularity, and a granulocyte-to-erythroid ratio of 10:1 to 30:1.⁶⁷ The increased cellularity is due to elevated myelopoiesis.⁶⁴ An increase in cellularity is also observed in other leukemia. In CML, basophilia in bone marrow is often observed.

An increase in megakaryocytopoietic activity with hypolobated nuclei and micromegakaryocytes is often observed in the bone marrow.⁶⁸ Examinations of bone marrows by Mughal et al showed the presence of megakaryocytic clustering in 60.6% of patients and found that megakaryocytic clustering was correlated with a high Sokal score.⁶⁹ Elevated reticulin fibrosis in the bone marrow has been observed in approximately 30% of CML patients and can be associated with worse symptoms; however, this has not been

Table 2 Baseline Diagnostic Workup Recommended by the ELN 2020, ESMO 2017, and NCCN 2021 Guidelines

ELN 2020	ESMO 2017	NCCN 2021
1. Physical examination with particular reference to spleen and liver size 2. Complete blood cell count with microscopic differential 3. Bone marrow aspirate for cytologic examination and cytogenetics; core biopsy if dry tap 4. Fluorescence in situ hybridization (FISH) only in the case of Ph negativity 5. Qualitative reverse-transcription polymerase chain reaction (PCR) for the detection of BCR-ABL1 transcripts and identification of the transcript type 6. Electrocardiogram 7. Standard biochemical profile with hepatitis B serology	1. Blood counts and differential 2. Bone marrow cytology 3. Bone marrow karyotype 4. Qualitative RT-PCR 5. Mutational analysis in the accelerated phase or blast phase	1. History and physical exam, including palpation of spleen 2. Complete blood count with differential 3. Chemistry profile 4. Hepatitis B panel 5. Bone marrow aspirate and biopsy for morphologic and cytogenetic evaluation 6. Quantitative reverse-transcription polymerase chain reaction (RT-PCR)

Notes: For additional information, readers should refer to CML guidelines by ELN 2020, ESMO 2017, and NCCN 2021. Adapted from *Ann Oncol*, 1(28), Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. iv41–51, Copyright 2017, with permission from Elsevier⁶⁴ and with adaptation from these other studies.^{38,65}

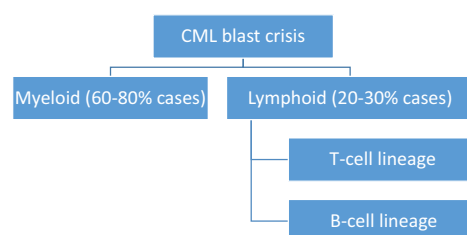
incorporated into the current CML prognostic stratification, similarly to megakaryocytic clustering.^{64,70} The blast proportion in the chronic phase should be <15%, and a number above this indicates progression to the accelerated phase or blast phase.⁶⁴ Immunohistochemistry through CD34 staining may be used to help blast identification.⁷¹

Cytogenetic analysis and qualitative PCR are recommended for CML diagnosis.^{38,64} In cytogenetic analysis, the Philadelphia chromosome may not be identified in some patients due to abnormal locations of the BCR-ABL1 gene. Hence, a FISH examination should be conducted to identify the location.³⁸ PCR examination of BCR-ABL transcript is the most reliable method of diagnosing CML. However, false-negative qualitative and quantitative PCR results may occur due to atypical BCR-ABL1 transcripts.⁶⁴

The accelerated CML phase occurs after one to three years of the chronic CML phase.¹ This phase can be suspected when there is an enlargement of the spleen that had shrunk previously following therapy, anemia that becomes more severe, or the development of petechiae or ecchymosis. The presence of a fever is usually a sign of infection. The majority of chronic CML patients will enter the accelerated phase before the blast phase.

The blast phase is characterized by symptoms of extramedullary blast infiltration in organs other than the liver and spleen, such as the lymph nodes, central nervous system, and lungs. Indeed, both the WHO and ELN guidelines use this symptom as one of the criteria for blast-phase diagnosis.^{38,72} A high blast percentage further differentiates this phase from the accelerated phase. According to the ELN 2020 CML guidelines, the blast percentage needed for diagnosis is $\geq 30\%$ in the peripheral blood or bone marrow.³⁸ This is in contrast with the WHO guideline, which state that a blast percentage of $\geq 20\%$ is needed.⁷² Overall, it is recommended to check for ACAs and gene mutations in the blast phase or when TKI treatment does not produce an adequate response. Furthermore, ACAs and gene mutations are useful for determining treatment.

The blast phase of CML resembles acute leukemia. The blast type is myeloid in up to 80% of cases, or lymphoid in up to 30% of cases (Figure 1).⁷³ In lymphoid blast crisis, B-cell lineage is much more common than T-cell lineage.^{74,75}

**Figure 1** Type of blast phase in CML.

Lymphoid blast crisis presents a unique diagnostic and therapeutic challenges. Readers are suggested to refer to an article by Yohannan et al for further information.²³

Therapies for CML

Chemotherapies

Busulfan was one of the earliest chemotherapies used for CML. In the past, busulfan was administered until leukocyte counts reduced to below 30×10^9 /L. As is the case for all chemotherapies, busulfan is associated with serious adverse effects. Those adverse effects include aplasia, pulmonary fibrosis, and severe infection.

Therapy with short-duration hydroxyurea can be given to CML patients with high leukocyte or high platelet counts with the aim of reducing leukocyte and/or platelet levels prior to the administration of a TKI.⁷⁶ The recommended duration of administration of hydroxyurea is not stated in the ELN's CML guideline. Another alternative to the use of hydroxyurea is apheresis (in certain conditions, such as hyperleukocytosis with leukostasis), but this is rarely used because the decrease in leukocytes following apheresis is only temporary. Nowadays, chemotherapies for CML have mostly been abandoned in favor of more effective therapies such as TKIs.

Interferon Alpha

Prior to the TKI era, the use of IFN α was dominant as a first-line therapy. Since the emergence of TKIs, IFN α has not been used as a first-line therapy because TKIs provide better survival and a better molecular response. However, several recent studies have shown that the combination of pegylated-IFN α (PEG-IFN α) with imatinib provides a faster and more potent molecular response.^{77,78} Currently, several clinical studies of the combination of PEG-IFN α with nilotinib or other TKIs are underway.

Stem Cell Therapy

The use of stem cell transplantation as an initial therapy has now been replaced by TKI therapy. Allogeneic stem cell transplantation may be considered for chronic CML patients who fail to respond to treatment with at least two TKIs. According to the European Society for Medical Oncology (ESMO) guidelines, chronic-stage CML patients who have a T315I mutation are also recommended for stem cell transplantation after a ponatinib trial.³⁸ Patients who are at high risk for transformation to the blast phase are candidates for stem cell transplantation because the outcomes of stem cell transplantation in the blast phase are poor.⁷⁹

Tyrosine Kinase Inhibitors

The first-line therapy for CML is a tyrosine kinase inhibitor (TKI), except in pregnant women. TKI therapy is a targeted therapy that inhibits the tyrosine kinase pathway in CML, which has been proven in clinical trials to provide better outcomes than previous CML treatments such as hydroxyurea, interferon alpha, and busulfan.

The first trial of a TKI in CML was imatinib compared with interferon and low-dose cytarabine in the newly diagnosed chronic-phase chronic myeloid leukemia (IRIS) study, which showed that 76.2% percent of CML patients given imatinib achieved a complete cytogenetic response (CCyR) after a median follow up of 19 months.⁸⁰ Meanwhile, in the cytarabine +IFN- α group, the CCyR only reached 14.5%. This striking difference indicated that imatinib provided significantly better CCyR outcomes in the CRF patients. In addition, imatinib provided better survival rates than previous therapies. Therefore, after the publication of this study, TKIs became the first-line therapy used for CML, replacing IFN- α and chemotherapy.

Imatinib works via competitive inhibition of the ATP-binding site in the kinase domain, which subsequently blocks the ability of BCR-ABL to phosphorylate tyrosine residues.⁸¹ Side effects of TKI treatment include hypertension, thrombosis, and pleural effusion.^{82,83} Currently, the mechanisms that cause these side effects are unknown and are the subject of ongoing investigation.

With continuous treatment, approximately 90% of CML patients treated with imatinib have a similar survival to with people without CML. However, the remaining 10% of patients display resistance to imatinib, shown by failure to achieve a molecular response; these patients can often be treated with a second-generation TKI such as dasatinib or nilotinib.³⁸ It should be noted that even with TKI treatments, there is a continuous presence of LSCs, as TKI usually does not eliminate

quiescent stem cells.⁵² A strategy that is currently being developed to eliminate LSCs is the combination of a TKI with thiazolidinediones. Thiazolidinediones, in a study by Prost et al, were shown to impair hematopoiesis through PPAR- γ -STAT5 signaling activation, which may be of benefit in destroying quiescent stem cells.⁸⁴

The second-generation TKIs consist of dasatinib, nilotinib, bosutinib, and radotinib. They tend to have a higher potency in inhibiting BCR-ABL1 mutants.⁸⁵ The DASISION trial showed that dasatinib 100 mg once daily was superior to imatinib 400 mg once daily in the outcomes of a MMR.⁸⁶ The outcome of MR4.5 was also higher from dasatinib than imatinib. The recent DASCERN study also showed a better MMR outcome from dasatinib.⁸⁷ Similarly, other second-generation TKIs (nilotinib, bosutinib, and radotinib) have higher MMR responses than imatinib.^{88–90}

Ponatinib is a third-generation TKI which has been shown to be more efficacious than other TKIs (Approximately 500 times more potent than imatinib) and is a TKI that has activity against T315I BCR-ABL1 point mutation.^{38,91,92} Recommended dose for ponatinib is 45 mg daily but this can be lowered into 30mg daily or 15 mg daily if side effects of thrombotic events, vascular occlusions, and heart failure are of major concern.^{1,38,93}

TKI Resistance

CML patients with TKI resistance should be treated with a second-line TKI and be examined for BCR-ABL1 mutations as the most common cause of TKI resistance is due to point mutations in the kinase domain of BCR-ABL1.⁹⁴ These point mutations cause a decrease binding affinity of TKI.⁹⁵ Recommended method to detect BCR-ABL1 resistance mutations is next generation sequencing (NGS).³⁸

The type of TKI given depends on the BCR-ABL1 resistance mutations found. For example, patients with a T315I mutation are recommended to be treated with ponatinib only as patients harboring this mutation have resistance to other TKIs.^{38,96} In contrast, patients with a Y253H, E255V/K, F359V/I/C mutations can be treated with dasatinib, bosutinib, or ponatinib.³⁸ ELN 2020 CML guideline has a list of recommended tyrosine kinase inhibitors for BCR-ABL1 resistance mutations.³⁸ In general, second-generation TKIs are recommended when there is BCR-ABL1 point mutations. The selection of second-generation or third-generation TKIs should be based on a balance between efficacy and side effects.¹

Treatment Response

The goal of therapy in CML is to achieve a hematological response, a cytogenetic response, and a long-term major molecular response. A complete hematological response (CHR) is defined as a normal leukocyte count, a normal platelet level, a normal differential result, and the disappearance of CML symptoms.⁹⁷ A partial hematological response is defined as a decrease in leukocyte count of less than 50% of the leukocyte level before treatment, or normalization of the leukocyte level with persistent splenomegaly or the presence of blasts in the peripheral blood. Meanwhile, a complete cytogenetic response (CCR) is defined as the absence of metaphase Ph+ in the bone marrow cells.⁹⁷

Currently, the major molecular response is more often used to monitor therapy, especially for TKI therapy.⁹⁷ Treatments such as chemotherapies and interferon alpha rarely achieve a major molecular response. Molecular responses should be quantified on an international scale using the ratio of BCR-ABL1 transcripts to ABL1 transcripts.⁹⁸ A molecular response of BCR-ABL1 1% is equivalent to that of CCR. The molecular response of BCR-ABL1 0.1% is defined as a major molecular response (MMR) or MR³. The molecular response of BCR-ABL1 0.01% is defined as MR⁴ (Table 3). There is also the term deep molecular response (DMR), defined as MR⁴ and above.⁹⁹ CD26, perhaps as a marker of LSCs, can be used as an alternative to detect response, especially for BCR-ABL-independent CML cells; however, it is also difficult to detect small numbers of CD26-positive LSCs.¹⁰⁰ Thus, currently, the monitoring of CML patients revolves around the molecular response.

Prognosis

There are currently four prognostic systems for CML: (1) Sokal, (2) Euro, (3) EUTOS, and (4) ELTS.^{101,102} However, it should be noted by clinicians that the Sokal score was developed in the chemotherapy era. Meanwhile, the Euro score was developed in the interferon alpha (IFN α) era. Thus, the eras in which these scoring systems were developed may reduce the validity of the Sokal and Euro scores, because almost all CML patients today are treated with a TKI. Indeed, it is now recommended that the EUTOS and ELTS scores be used instead.

Table 3 BCR-ABL1 International Scale and Molecular Response

BCR-ABL1 International Scale	Log Reduction	Molecular Response	Categories
10	1	MCyR	Non-Deep Molecular Response
1	2	CCyR	
0.1	3	MMR	
0.01	4	MR ⁴	Deep Molecular Response
0.0032	4.5	MR ^{4.5}	
0.0001	5	MR ⁵	
0.00001	6	MR ⁶	

Abbreviations: MCyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response.

The European treatment and outcome study (EUTOS) scoring system predicts CCR at 18 months after TKI therapy. In contrast to other scoring systems, the EUTOS long-term survival (ELTS) score predicts the mortality risk of CML patients given a TKI therapy using the same variables as the Sokal scoring system. Currently, the ELN recommends the use of the ELTS scoring system compared to the other scoring systems.³⁸

Treatment-Free Remission

Previously, the aim of CML therapy was to delay progression to the blast phase, minimize symptoms, and increase overall survival. These aims are mostly achieved in most patients today. Hence, new outcomes for CML patients, such as treatment-free remission with TKI discontinuation, are now being pursued, especially since there are concerns due to the long-term toxicity of TKI therapies. Another important reason is the cost associated with TKI therapy. Currently, research on CML is focused on achieving DMR to achieve treatment-free remission (TFR).

In general, CML patients require lifelong therapy to prevent recurrence, even when the patient has achieved an MMR status for several years; however, this trend is now changing. The antiproliferative effects of TKIs on LSCs have been shown to be strong; however, the effect on apoptosis is weaker.¹⁰³ The reason for this is that quiescent LSCs are resistant to apoptosis due to TKIs. A further problem is associated with the BCR-ABL-independent pathway, which maintains the survival of CML stem cells. A study by Jeanpierre et al showed that quiescent stem cells activated BMP1B, Stat3, and BMP4-niche signals to maintain their survival.¹⁰⁴ These signals may not be targeted by TKIs, especially early-generation TKIs.

Recently, it has become known that CML patients who successfully achieve a long-lasting DMR can achieve complete recovery. Such patients have a lower risk of CML relapse when TKI therapy is discontinued. However, only a small proportion of CML patients can achieve a DMR status with their current therapy, due to the presence of CML stem cells left over after therapy. Several trials have shown that a significant proportion of CML patients (approximately 60%) with undetectable minimal residual disease still have a relapse, shown by the identification of BCFR-ABL1-positive cells within the first 2 years after imatinib discontinuation.^{7,105,106}

Based on the results of the stop imatinib (STIM) clinical trial, with a median follow-up of 77 months, 39% of CML patients who achieved MR⁴ status for two years were able to maintain their remission status without TKI therapy.¹⁰⁵ A meta-analysis by Campiotti et al (2017) of 15 cohort studies of patients with undetectable BCR-ABL1 transcript criteria showed that the mean molecular recurrence rate was 51% (95% CI 44–58%; I²: 55%).¹⁰⁷ The percentage of TFR in patients who achieved an MMR alone without a DMR was lower than that in patients who achieved a DMR. This result can be interpreted as showing that a significant proportion of patients may achieve TFR.

From all of the available studies, it can be inferred that only CML patients who achieve DMR are candidates for treatment-free remission (Table 4). The factors associated with successful TFR have not yet been established. However, a study by Irani et al demonstrated that CML patients who achieved TFR had higher levels of natural killer (NK) cells.¹⁰⁸ This finding was similar to the results of a sub-analysis of EURO-SKI.¹⁰⁹ It should be noted that even in patients who

Table 4 Summary of TFR Studies

Trials	Year of Publication	Country	Sample Size (n)	TKI Used	Minimum TKI Treatment Duration	DMR Definition Used	Minimum DMR Duration	Results of TFR Rate After Stopping Treatment
5-year update of the ENESTfreedom trial ¹¹⁰	2021	Multiple countries	190	Nilotinib	2 years	MR ^{4,5}	1 year	42.6% at 5 years
ISAV ¹¹¹	2020	Italy	107	Imatinib	2 years	UMRD	18 months	48% at 36 months
DADI (2020) ¹¹²	2020	Japan	58	Dasatinib	3 years	DMR	2 years	55% at 6 months
Fava et al ¹¹³	2019	Italy	293	Imatinib, Nilotinib, dasatinib, bosutinib	7 years	MR ⁴	Median of 46 months (IQR 31;74)	62% (95% CI: 56;68) at 34 months; 68% (95% CI: 62;74) for imatinib, 73% (95% CI: 64;83) for second generation TKI
Pagnano KB ¹¹⁴	2019	Brazil	48	Imatinib	3 years	MR ⁴	2 years	61% (95% CI 47–75) at 20 months
DASFREE ¹¹⁵	2019	USA	84	Dasatinib	2 years	MR ^{4,5}	1 year	46% at 2 years
NILS ¹¹⁶	2019	Japan	87	Imatinib, nilotinib	2 years	MR ^{4,5}	2 years	60.9% (90% CI 51.6–69.7)
EURO-SKI ¹¹⁷	2018	Europe	755	Imatinib, nilotinib, dasatinib	3 years	MR ⁴	1 year	50% (95% CI 46–54) at 24 months
JALSG-STIM231 ¹¹⁸	2018	Japan	68	Imatinib	3 years	MR ⁴	2 years	67.6% at 12 months
D-STOP ¹¹⁹	2018	Japan	54	Dasatinib	2 years	DMR	2 years	62.9% (95% confidence interval: 48.5%–74.2%) at 12 months
STAT2 ¹²⁰	2018	Japan	78	Nilotinib	2 years	MR ^{4,5}	2 years	62.8% (95% CI: 51.1–73.5%) at 24 months
DOMEST ¹²¹	2018	Japan	99	Imatinib	2 years	MR ⁴	2 years	64.3% at 24 months
Hernandez-Boluda et al ¹²²	2018	Spain	236	Imatinib, Nilotinib, Dasatinib, Bosutinib, Ponatinib	3 years	MR ^{4,5}	2 years	64% (95% CI: 55–72) at 4 years
Mahon ¹²³	2018	Multiple countries	126	Imatinib, nilotinib	3 years	MR ^{4,5}	1 year	53% (95% CI: 44% to 62%) at 96 weeks

(Continued)

Table 4 (Continued).

Trials	Year of Publication	Country	Sample Size (n)	TKI Used	Minimum TKI Treatment Duration	DMR Definition Used	Minimum DMR Duration	Results of TFR Rate After Stopping Treatment
Long-term STIM1 ⁷	2017	France	100	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	38% (95% CI, 29% to 47%) at 60 months
ENESTfreedom ¹²⁴	2017	Multiple countries	190	Nilotinib	2 years	MR ^{4.5}	1 year	51.65 (95% CI: 44.2–58.9) at 48 weeks
STOP 2G ¹²⁵	2017	France	60	Nilotinib, dasatinib	3 years	MR ^{4.5}	2 years	53.57% (95% CI, 40.49% to 66.65%) at 48 months
KID ¹⁰⁶	2016	Korea	90	Imatinib	3 years	MR ^{4.5}	2 years	58.5% ± 5.2% at 24 months
TRAD ¹²⁶	2016	Canada	67	Imatinib, dasatinib	3 years	MR ^{4.5}	2 years	64.7% (48.7–76.7%) at 6 months
DADI (2015) ¹²⁷	2015	Japan	63	Dasatinib	1 year	DMR	3 years	49% (95% CI 36–61) at 6 months
TWISTER ¹²⁸	2013	Australia	40	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	47.1% at 24 months
ASTIM ¹²⁹	2013	France	80	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	61% (95% CI, 51% to 73%) at 36 months
HOVON ¹³⁰	2013	Netherlands	15	Imatinib	2 years	MR ^{4.5}	2 years	33% (CI 44–88%) at 24 months
STIM2 ¹³¹	2013	France	124	Imatinib	3 years	DMR	2 years	61.2% at 12 months
Ho-Young Yhimetal ¹³²	2012	South Korea	14	Imatinib	2 years	DMR	Unknown	28.6% at 12 months
Kieo STIM ¹³³	2012	Japan	40	Imatinib	2 years	DMR	2 years	55.4% at 12 months
STIM1 ¹⁰⁵	2010	France	100	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	41% at 24 months

achieve a DMR, LSCs are not removed completely. It can be speculated that the patients that achieve TFR have an adequate immunological response in suppressing residual LSCs.

Predictors and Biomarkers for TFR

Clinical parameters and biomarkers that can be used to predict TFR in CML patients are still under investigation and this remains a topic of interest (Figure 2). Vigón et al identified several immunological parameters that predict relapse.¹³⁴ These parameters are low levels of natural killer cells, natural killer T cells, and CD8⁺TCR $\gamma\beta$ ⁺ T cells.¹³⁴ Similarly, Rea et al found that higher number of natural killer (NK) cells were observed in CML patients that did not relapse.¹³⁵ Both CD26 and CD93 which are circulating CML stem cell markers appear to be correlated with TFR as well.^{51,136,137} Other possible markers include regulatory T cells, cytotoxic CD8⁺ T lymphocyte (CTL), and somatic mutations.^{134,138–140} Type of BCR-ABL1 transcript also has a role; in a study by D'Adda et al, TFR maintenance was observed to be higher in CML patients with e14a2 transcript when compared with e13a2 transcripts.¹⁴¹ However, all of these biomarkers are not readily available for routine clinical use, and thus, clinical parameters remain the most widely used or accessible parameters for TFR.¹⁴²

Many studies have data that can be used to identify clinical parameters that can be used for TFR predictions.^{115,143–145} In general, longer duration of TKI therapy, longer DMR duration, older age, undetectable BCR-ABL1 3 months after TKI discontinuation, and deeper DMR status are observed as predictors for TFR.^{115,143,144,146,147} Recent study by Kim et al proposed six years imatinib treatment and 4.5 years of MR⁴ response as the optimal duration for starting TFR.¹⁴⁷

It is speculated that adding concomitant ruxolitinib or pembrolizumab to patients' TKI therapy may produce a beneficial outcome.¹⁴⁸ In the future, it is hoped that treatment-free remission can become more achievable for CML patients and can also become a treatment outcome for other cancers as well.

Side Effects of TKI Discontinuation

Several long-term CML patients who have been treated with a TKI have had side effects when discontinuing their TKI. Side effects of TKI discontinuation include mostly muscle and joint pain. These side effects can last up to a few months and can be treated with supportive care such as NSAIDs. Around 30% of patients may experience musculoskeletal pain after TKI discontinuation.^{143,149,150} Musculoskeletal pain may be experienced in different regions of the body such as the

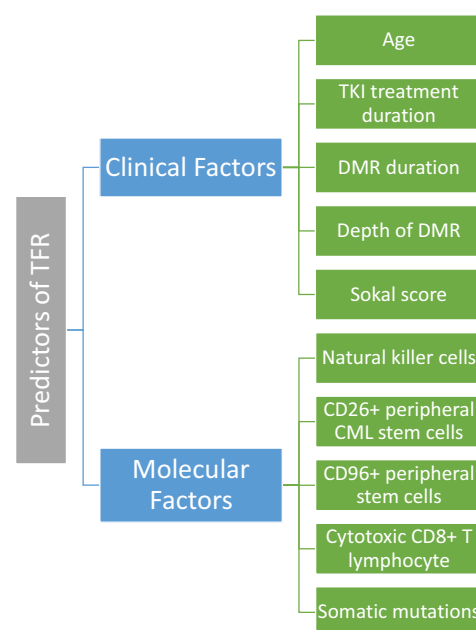


Figure 2 Known predictors of treatment-free remission.

shoulders, extremities, hips, hands, and feet.¹⁵⁰ A study by Hernandez-Boluda et al revealed that patients that stopped TKI had elevated cholesterol level after discontinuing with the exception of nilotinib.¹²²

Currently, the mechanisms of TKI withdrawal symptoms are unclear.¹⁰⁶ It is speculated that TKIs inhibit c-kit and platelet-derived growth factor receptor (PDGFR), which results in an increased number of osteoclasts and osteoblasts.^{149,150} A small study by Katagiri et al found that patients with symptoms of musculoskeletal pain after discontinuing TKI treatment had a lower body weight and lower BMI, but other factors that can predict whether patients will develop musculoskeletal pain are still unknown.¹⁴⁹

Generally, the side effects of TKI discontinuation are not a major concern, as most patients' symptoms resolve within 3 months and most of the symptoms are mild.¹⁵¹ However, clinicians should be aware of the side effects and educate patients as needed to prepare them for this possibility.

Conclusion

Many advances have been made in improving our understanding of the pathophysiological processes of CML. However, the etiology of the Philadelphia chromosome and mechanisms of LSC persistence are still under investigation. Somatic mutations and additional chromosomal abnormalities are associated with CML progression into blast phase. The frontline treatment of CML currently is TKI as it is more effective than previous therapies. The current aim of CML treatment is to achieve TFR, and CML patients who achieve a DMR and receive long-term TKI therapy have a higher likelihood of achieving this. In the future, it is hoped that more patients can achieve treatment-free remission.

Acknowledgement

The authors would like to express their gratitude to Yehezkiel Alexander Eduard George, MD, for his professional assistance in proofreading the galley proof of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2020 update on diagnosis, therapy and monitoring. *Am J Hematol*. 2020;95(6):691–709. doi:10.1002/ajh.25792
2. Nicholson E, Holyoake T. The chronic myeloid leukemia stem cell. *Clin Lymphoma Myeloma*. 2009;9(Suppl 4):S376–381. doi:10.3816/CLM.2009.s.037
3. Frazer R, Irvine AE, McMullin MF. Chronic myeloid leukaemia in the 21st century. *Ulster Med J*. 2007;76(1):8–17.
4. García-Gutiérrez V, Hernández-Boluda JC. Tyrosine kinase inhibitors available for chronic myeloid leukemia: efficacy and safety. *Front Oncol*. 2019;9. doi:10.3389/fonc.2019.00603
5. Sasaki K, Strom SS, O'Brien S, et al. Relative survival in patients with chronic-phase chronic myeloid leukaemia in the tyrosine-kinase inhibitor era: analysis of patient data from six prospective clinical trials. *Lancet Haematol*. 2015;2(5):e186–193. doi:10.1016/S2352-3026(15)00048-4
6. Hochhaus A, Larson RA, Guilhot F, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med*. 2017;376(10):917–927. doi:10.1056/NEJMoa1609324
7. Etienne G, Guilhot J, Rea D, et al. Long-term follow-up of the French stop imatinib (STIM1) study in patients with chronic myeloid leukemia. *J Clin Oncol off J Am Soc Clin Oncol*. 2017;35(3):298–305. doi:10.1200/JCO.2016.68.2914
8. Oka S, Muroi K, Mori M, et al. Prediction of response to imatinib in patients with chronic myelogenous leukemia by flow cytometric analysis of bone marrow blastic cell phenotypes. *Leuk Lymphoma*. 2009;50(2):290–293. doi:10.1080/10428190802627598
9. Kang ZJ, Liu YF, Xu LZ, et al. The Philadelphia chromosome in leukemogenesis. *Chin J Cancer*. 2022;35:48. doi:10.1186/s40880-016-0108-0
10. Holmberg M. Is the primary event in radiation-induced chronic myelogenous leukemia the induction of the t(9;22) translocation? *Leuk Res*. 1992;16(4):333–336. doi:10.1016/0145-2126(92)90134-S
11. Ismail SI, Naffa RG, Yousef AMF, Ghanim MT. Incidence of bcr-abl fusion transcripts in healthy individuals. *Mol Med Rep*. 2014;9(4):1271–1276. doi:10.3892/mmr.2014.1951
12. Aplan PD. Causes of oncogenic chromosomal translocation. *Trends Genet TIG*. 2006;22(1):46–55. doi:10.1016/j.tig.2005.10.002
13. Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood*. 2022;113(8):1619–1630. doi:10.1182/blood-2008-03-144790
14. Verma D, Kantarjian HM, Jones D, et al. Chronic myeloid leukemia (CML) with P190 BCR-ABL: analysis of characteristics, outcomes, and prognostic significance. *Blood*. 2009;114(11):2232–2235. doi:10.1182/blood-2009-02-204693
15. Arun AK, Senthamizhselvi A, Mani S, et al. Frequency of rare BCR-ABL1 fusion transcripts in chronic myeloid leukemia patients. *Int J Lab Hematol*. 2017;39(3):235–242. doi:10.1111/ijlh.12616

16. Cortes JE, Talpaz M, Beran M, et al. Philadelphia chromosome-negative chronic myelogenous leukemia with rearrangement of the breakpoint cluster region. Long-term follow-up results. *Cancer*. 1995;75(2):464–470. doi:10.1002/1097-0142(19950115)75:2<464::AID-CNCR2820750209>3.0.CO;2-E
17. Seong D, Kantarjian HM, Albitar M, et al. Analysis of Philadelphia chromosome-negative BCR-ABL-positive chronic myelogenous leukemia by hypermetaphase fluorescence in situ hybridization. *Ann Oncol off J Eur Soc Med Oncol*. 1999;10(8):955–959. doi:10.1023/A:1008349405763
18. Onida F, Ball G, Kantarjian HM, et al. Characteristics and outcome of patients with Philadelphia chromosome negative, bcr/abl negative chronic myelogenous leukemia. *Cancer*. 2002;95(8):1673–1684. doi:10.1002/cncr.10832
19. Ohyashiki JH, Ohyashiki K, Ito H, Toyama K. Molecular and clinical investigations in Philadelphia chromosome-negative chronic myelogenous leukemia. *Cancer Genet Cytogenet*. 1988;33(1):119–126. doi:10.1016/0165-4608(88)90057-X
20. Wang SA, Hasserjian RP, Fox PS, et al. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. *Blood*. 2014;123(17):2645–2651. doi:10.1182/blood-2014-02-553800
21. Diamantopoulos PT, Viniou NA. Atypical chronic myelogenous leukemia, BCR-ABL1 negative: diagnostic criteria and treatment approaches. *Front Oncol*. 2021;11:722507. doi:10.3389/fonc.2021.722507
22. Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest*. 2010;120(7):2254–2264. doi:10.1172/JCI41246
23. Yohannan B, George B. B-lymphoid blast phase–chronic myeloid leukemia: current therapeutics. *Int J Mol Sci*. 2022;23(19):11836. doi:10.3390/ijms231911836
24. Nowicki MO, Falinski R, Koptyra M, et al. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. *Blood*. 2004;104(12):3746–3753. doi:10.1182/blood-2004-05-1941
25. Koptyra M, Falinski R, Nowicki MO, et al. BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood*. 2006;108(1):319–327. doi:10.1182/blood-2005-07-2815
26. Skorski T. Oncogenic tyrosine kinases and the DNA-damage response. *Nat Rev Cancer*. 2002;2(5):351–360. doi:10.1038/nrc799
27. Chereda B, Melo JV. Natural course and biology of CML. *Ann Hematol*. 2015;94(Suppl 2):107–121. doi:10.1007/s00277-015-2325-z
28. Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood*. 2009;113(8):1619–1630. doi:10.1182/blood-2008-03-144790
29. How HR. I treat CML blast crisis. *Blood*. 2012;120(4):737–747. doi:10.1182/blood-2012-03-380147
30. Adnan Awad S, Dufva O, Ianevski A, et al. RUNX1 mutations in blast-phase chronic myeloid leukemia associate with distinct phenotypes, transcriptional profiles, and drug responses. *Leukemia*. 2021;35(4):1087–1099. doi:10.1038/s41375-020-01011-5
31. Ochi Y, Yoshida K, Huang YJ, et al. Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. *Nat Commun*. 2021;12(1):2833. doi:10.1038/s41467-021-23097-w
32. Soliman DS, Amer AM, Mudawi D, et al. Chronic myeloid leukemia with cryptic Philadelphia translocation and extramedullary B-lymphoid blast phase as an initial presentation. *Acta Bio*. 2018;89(3–S):38–44.
33. Zagaria A, Anelli L, Albano F, et al. A fluorescence in situ hybridization study of complex t(9;22) in two chronic myelocytic leukemia cases with a masked Philadelphia chromosome. *Cancer Genet Cytogenet*. 2004;150(1):81–85. doi:10.1016/j.cancergencyto.2003.08.018
34. Alhurajji A, Kantarjian H, Boddu P, et al. Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. *Am J Hematol*. 2018;93(1):84–90. doi:10.1002/ajh.24943
35. Krishna Chandran R, Geetha N, Sakthivel KM, Suresh Kumar R, Jagathnath Krishna KMN, Sreedharan H. Impact of additional chromosomal aberrations on the disease progression of chronic myelogenous leukemia. *Front Oncol*. 2019;5(9):88. doi:10.3389/fonc.2019.00088
36. Chen Z, Shao C, Wang W, et al. Cytogenetic landscape and impact in blast phase of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. *Leukemia*. 2017;31(3):585–592. doi:10.1038/leu.2016.231
37. Clark RE, Apperley JF, Copland M, Cicconi S. Additional chromosomal abnormalities at chronic myeloid leukemia diagnosis predict an increased risk of progression. *Blood Adv*. 2021;5(4):1102–1109. doi:10.1182/bloodadvances.2020003570
38. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*. 2020;34(4):966–984. doi:10.1038/s41375-020-0776-2
39. Meggyesi N, Kozma A, Halm G, Nahajevsky S, Bártai A, Fekete S, et al. Additional chromosome abnormalities, BCR-ABL tyrosine kinase domain mutations and clinical outcome in Hungarian tyrosine kinase inhibitor-resistant chronic myelogenous leukemia patients. *Acta Haematol*. 2012;127(1):34–42.
40. Siti Mariam I, Norhidayah R, Zulaikha AB, et al. Differential prognostic impact of stratified additional chromosome abnormalities on disease progression among Malaysian chronic myeloid leukemia patients undergoing treatment with imatinib mesylate. *Front Oncol*. 2022;12. doi:10.3389/fonc.2022.720845
41. Houshmand M, Simonetti G, Circosta P, et al. Chronic myeloid leukemia stem cells. *Leukemia*. 2019;33(7):1543–1556. doi:10.1038/s41375-019-0490-0
42. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia*. 2007;21(5):926–935. doi:10.1038/sj.leu.2404609
43. Kavalierchik E, Goff D, Jamieson CHM. Chronic myeloid leukemia stem cells. *J Clin Oncol off J Am Soc Clin Oncol*. 2008;26(17):2911–2915. doi:10.1200/JCO.2008.17.5745
44. Herrmann H, Cerny-Reiterer S, Gleixner KV, et al. CD34(+)/CD38(-) stem cells in chronic myeloid leukemia express Siglec-3 (CD33) and are responsive to the CD33-targeting drug gemtuzumab/ozogamicin. *Haematologica*. 2012;97(2):219–226. doi:10.3324/haematol.2010.035006
45. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells – what challenges do they pose? *Nat Rev Drug Discov*. 2014;13(7):497–512. doi:10.1038/nrd4253
46. Minami Y, Kono T, Miyazaki T, Taniguchi T. The IL-2 receptor complex: its structure, function, and target genes. *Annu Rev Immunol*. 1993;11:245–268. doi:10.1146/annurev.iy.11.040193.001333
47. Sadovnik I, Herrmann H, Eisenwort G, et al. Expression of CD25 on leukemic stem cells in BCR-ABL1+ CML: potential diagnostic value and functional implications. *Exp Hematol*. 2022;51:17–24. doi:10.1016/j.exphem.2017.04.003
48. Herrmann H, Sadovnik I, Cerny-Reiterer S, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. *Blood*. 2014;123(25):3951–3962. doi:10.1182/blood-2013-10-536078

49. Gschwandtner M, Paulitschke V, Mildner M, et al. Proteome analysis identifies L1CAM/CD171 and DPP4/CD26 as novel markers of human skin mast cells. *Allergy*. 2017;72(1):85–97. doi:10.1111/all.12919
50. Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc Natl Acad Sci U S A*. 2007;104(47):18660–18665. doi:10.1073/pnas.0705939104
51. Kinstrie R, Home GA, Morrison H, et al. CD93 is expressed on chronic myeloid leukemia stem cells and identifies a quiescent population which persists after tyrosine kinase inhibitor therapy. *Leukemia*. 2020;34(6):1613–1625. doi:10.1038/s41375-019-0684-5
52. Chu S, McDonald T, Lin A, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood*. 2022;118(20):5565–5572. doi:10.1182/blood-2010-12-327437
53. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. 2009;458(7239):776–779. doi:10.1038/nature07737
54. Chen Y, Hu Y, Zhang H, Peng C, Li S. Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia. *Nat Genet*. 2009;41(7):783–792. doi:10.1038/ng.389
55. Kuepper MK, Bütow M, Herrmann O, et al. Stem cell persistence in CML is mediated by extrinsically activated JAK1-STAT3 signaling. *Leukemia*. 2019;33(8):1964–1977. doi:10.1038/s41375-019-0427-7
56. Holyoake TL, Vetrie D. The chronic myeloid leukemia stem cell: stemming the tide of persistence. *Blood*. 2017;129(12):1595–1606. doi:10.1182/blood-2016-09-696013
57. Skorski T. BCR/ABL, DNA damage and DNA repair: implications for new treatment concepts. *Leuk Lymphoma*. 2008;49(4):610–614. doi:10.1080/03093640701859089
58. Stoklosa T, Poplawski T, Koptyra M, et al. BCR/ABL inhibits mismatch repair to protect from apoptosis and induce point mutations. *Cancer Res*. 2008;68(8):2576–2580. doi:10.1158/0008-5472.CAN-07-6858
59. Cramer K, Nieborowska-Skorska M, Koptyra M, et al. BCR/ABL and other kinases from chronic myeloproliferative disorders stimulate single-strand annealing, an unfaithful DNA double-strand break repair. *Cancer Res*. 2008;68(17):6884–6888. doi:10.1158/0008-5472.CAN-08-1101
60. Zhang B, Chu S, Agarwal P, et al. Inhibition of interleukin-1 signaling enhances elimination of tyrosine kinase inhibitor-treated CML stem cells. *Blood*. 2016;128(23):2671–2682. doi:10.1182/blood-2015-11-679928
61. Bhatia R. TARGETING LEUKEMIA STEM CELL RESISTANCE IN CHRONIC MYELOGENOUS LEUKEMIA. *Trans Am Clin Climatol Assoc*. 2019;130:246–254.
62. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol*. 2020;13(1):104. doi:10.1186/s13045-020-00937-8
63. Gallipoli P, Cook A, Rhodes S, et al. JAK2/STAT5 inhibition by nilotinib with ruxolitinib contributes to the elimination of CML CD34+ cells in vitro and in vivo. *Blood*. 2014;124(9):1492–1501. doi:10.1182/blood-2013-12-545640
64. Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*. 2017;1(28):iv41–51. doi:10.1093/annonc/mdx219
65. Deininger MW, Shah NP, Altman JK, et al. Chronic myeloid leukemia, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw JNCCN*. 2020;18(10):1385–1415. doi:10.6004/jnccn.2020.0047
66. Asnafi AA, Deris Zayeri Z, Shahrabi S, Zibara K, Vosughi T. Chronic myeloid leukemia with complex karyotypes: prognosis and therapeutic approaches. *J Cell Physiol*. 2019;234(5):5798–5806. doi:10.1002/jcp.27505
67. ClinicalKey. Chronic myeloid leukemia; 2022. Available from: <https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323357623000676?scrollTo=%23hl0000264>. Accessed February 10, 2023.
68. Arunachalam AK, Jain M, Kumar A, Kushwaha R, Singh US, Tripathi AK. Megakaryocytes in chronic phase of chronic myeloid leukemia: a descriptive case series. *Ann Pathol Lab Med*. 2016;3(3):A176–182.
69. Mughal Z, Babar H, Ashraf S, Hamid A, Khokhar A, Qamar S. Megakaryocytic clustering in chronic myeloid leukemia: can it be a predictor of clinical outcome? *J Coll Physicians Surg*. 2021;31(1):34–38. doi:10.29271/jcpsp.2021.01.34
70. Dekmezian R, Kantarjian HM, Keating MJ, Talpaz M, McCredie KB, Freireich EJ. The relevance of reticulin stain-measured fibrosis at diagnosis in chronic myelogenous leukemia. *Cancer*. 1987;59(10):1739–1743. doi:10.1002/1097-0142(19870515)59:10<1739::AID-CNCR2820591011>3.0.CO;2-2
71. Haznedaroğlu İC, Kuzu I, İlhan O. WHO 2016 definition of chronic myeloid leukemia and tyrosine kinase inhibitors. *Turk J Hematol*. 2020;37(1):42–47.
72. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–2405. doi:10.1182/blood-2016-03-643544
73. Ivanov S, Sharma P, Jobanputra Y, Zhang Y. Transformation of chronic myeloid leukemia to acute biphenotypic leukemia. *J Med Cases*. 2020;11(8):239–242. doi:10.14740/jmc3511
74. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the world health organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375–2390. doi:10.1182/blood-2016-01-643569
75. Padhi P, Topalovski M, El Behery R, Cantu ES, Medavarapu R, Rare A. Case of chronic myelogenous leukemia presenting as T-cell lymphoblastic crisis. *Case Rep Oncol Med*. 2018;2018:7276128. doi:10.1155/2018/7276128
76. Andretta E, Costa C, Longobardi C, et al. Potential approaches versus approved or developing chronic myeloid leukemia therapy. *Front Oncol*. 2022;11:801779. doi:10.3389/fonc.2021.801779
77. Simonsson B, Gedde-Dahl T, Markevörn B, et al. Combination of pegylated IFN-α2b with imatinib increases molecular response rates in patients with low- or intermediate-risk chronic myeloid leukemia. *Blood*. 2011;118(12):3228–3235. doi:10.1182/blood-2011-02-336685
78. Preudhomme C, Guilhot J, Nicolini FE, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med*. 2010;363(26):2511–2521. doi:10.1056/NEJMoa1004095
79. Gratwohl A, Pfirrmann M, Zander A, et al. Long-term outcome of patients with newly diagnosed chronic myeloid leukemia: a randomized comparison of stem cell transplantation with drug treatment. *Leukemia*. 2016;30(3):562–569. doi:10.1038/leu.2015.281
80. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(11):994–1004. doi:10.1056/NEJMoa022457
81. Iqbal N, Iqbal N. Imatinib: a breakthrough of targeted therapy in cancer. *Chemother Res Pract*. 2022;2022:357027.
82. Uzer E, Ünal A, Köker MY, Doğan SA. The Side Effects of Imatinib. *Turk J Hematol*. 2022;30(3):341. doi:10.4274/TJH-2011.0018

83. Mughal TI, Schrieber A. Principal long-term adverse effects of imatinib in patients with chronic myeloid leukemia in chronic phase. *Biol Targets Ther.* 2010;2(4):315–323. doi:10.2147/BTT.S5775
84. Prost S, Le Dantec M, Augé S, et al. Human and simian immunodeficiency viruses deregulate early hematopoiesis through a Nef/PPARgamma/STAT5 signaling pathway in macaques. *J Clin Invest.* 2008;118(5):1765–1775. doi:10.1172/JCI33037
85. Breccia M, Alimena G. Second-Generation Tyrosine Kinase Inhibitors (TKi) as salvage therapy for resistant or intolerant patients to prior TKIs. *Mediterr J Hematol Infect Dis.* 2014;6(1):e2014003. doi:10.4084/mjhid.2014.003
86. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. *J Clin Oncol off J Am Soc Clin Oncol.* 2016;34(20):2333–2340. doi:10.1200/JCO.2015.64.8899
87. Cortes JE, Jiang Q, Wang J, et al. Dasatinib vs. imatinib in patients with chronic myeloid leukemia in chronic phase (CML-CP) who have not achieved an optimal response to 3 months of imatinib therapy: the DASCERN randomized study. *Leukemia.* 2020;34(8):2064–2073. doi:10.1038/s41375-020-0805-1
88. Hochhaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. *Leukemia.* 2016;30(5):1044–1054. doi:10.1038/leu.2016.5
89. Cortes JE, Gambacorti-Passerini C, Deininger MW, et al. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. *J Clin Oncol off J Am Soc Clin Oncol.* 2018;36(3):231–237. doi:10.1200/JCO.2017.74.7162
90. Do YR, Kwak JY, Kim JA, et al. Long-term data from a Phase 3 study of radotinib versus imatinib in patients with newly diagnosed, chronic myeloid leukaemia in the chronic phase (RERISE). *Br J Haematol.* 2020;189(2):303–312. doi:10.1111/bjh.16381
91. O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell.* 2009;16(5):401–412. doi:10.1016/j.ccr.2009.09.028
92. Zhou T, Commodore L, Huang WS, et al. Structural mechanism of the Pan-BCR-ABL inhibitor ponatinib (AP24534): lessons for overcoming kinase inhibitor resistance. *Chem Biol Drug Des.* 2011;77(1):1–11. doi:10.1111/j.1747-0285.2010.01054.x
93. Chan O, Talati C, Isenalmhe L, et al. Side-effects profile and outcomes of ponatinib in the treatment of chronic myeloid leukemia. *Blood Adv.* 2020;4(3):530–538. doi:10.1182/bloodadvances.2019000268
94. Alves R, Gonçalves AC, Rutella S, et al. Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia—from molecular mechanisms to clinical relevance. *Cancers.* 2021;13(19):4820. doi:10.3390/cancers13194820
95. Meenakshi Sundaram DN, Jiang X, Brandwein JM, Valencia-Serna J, Remant KC, Uludağ H. Current outlook on drug resistance in chronic myeloid leukemia (CML) and potential therapeutic options. *Drug Discov Today.* 2019;24(7):1355–1369. doi:10.1016/j.drudis.2019.05.007
96. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A Phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med.* 2013;369(19):1783–1796. doi:10.1056/NEJMoa1306494
97. Galinsky I, Buchanan S. Guide to interpreting disease responses in chronic myeloid leukemia. *J Adv Pract Oncol.* 2022;3(4):225–236.
98. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood.* 2006;108(6):1809–1820. doi:10.1182/blood-2006-02-005686
99. Branford S. Why is it critical to achieve a deep molecular response in chronic myeloid leukemia? *Haematologica.* 2022;105(12):2730–2737. doi:10.3324/haematol.2019.240739
100. Ebian HF, Abdelnabi ASM, Abdelazem AS, Khamis T, Fawzy HM, Hussein S. Peripheral blood CD26 positive leukemic stem cells as a possible diagnostic and prognostic marker in chronic myeloid leukemia. *Leuk Res Rep.* 2022;17:100321. doi:10.1016/j.lrr.2022.100321
101. Kuntegowdanahalli LC, Kanakasetty GB, Thanky AH, et al. Prognostic and predictive implications of Sokal, Euro and EUTOS scores in chronic myeloid leukaemia in the imatinib era—experience from a tertiary oncology centre in Southern India. *Ecancermedicalscience.* 2022;10:679.
102. Pfirrmann M, Clark RE, Prejzner W, et al. The EUTOS long-term survival (ELTS) score is superior to the Sokal score for predicting survival in chronic myeloid leukemia. *Leukemia.* 2022;34(8).
103. Munje C, Copland M. Exploring stem cell heterogeneity in chronic myeloid leukemia. *Trends Cancer.* 2022;4(3):167–169. doi:10.1016/j.trecan.2017.12.001
104. Jeanpierre S, Arizkane K, Thongjuea S, et al. The quiescent fraction of chronic myeloid leukemic stem cells depends on BMPR1B, Stat3 and BMP4-niche signals to persist in patients in remission. *Haematologica.* 2022;106(1):111–122. doi:10.3324/haematol.2019.232793
105. Mahon FX, Réa D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol.* 2010;11(11):1029–1035. doi:10.1016/S1470-2045(10)70233-3
106. Lee SE, Choi SY, Song HY, et al. Imatinib withdrawal syndrome and longer duration of imatinib have a close association with a lower molecular relapse after treatment discontinuation: the KID study. *Haematologica.* 2016;101(6):717–723. doi:10.3324/haematol.2015.139899
107. Campiotti L, Suter MB, Guasti L, et al. Imatinib discontinuation in chronic myeloid leukaemia patients with undetectable BCR-ABL transcript level: a systematic review and a meta-analysis. *Eur J Cancer Oxf Engl.* 2017;77:48–56.
108. Irani YD, Hughes A, Clarkson J, et al. Successful treatment-free remission in chronic myeloid leukaemia and its association with reduced immune suppressors and increased natural killer cells. *Br J Haematol.* 2020;191(3):433–441. doi:10.1111/bjh.16718
109. Ilander M, Olsson-Strömberg U, Schlums H, et al. Increased proportion of mature NK cells is associated with successful imatinib discontinuation in chronic myeloid leukemia. *Leukemia.* 2017;31(5):1108–1116. doi:10.1038/leu.2016.360
110. Radich JP, Hochhaus A, Masszi T, et al. Treatment-free remission following frontline nilotinib in patients with chronic phase chronic myeloid leukemia: 5-year update of the ENESTfreedom trial. *Leukemia.* 2021;35(5):1344–1355. doi:10.1038/s41375-021-01205-5
111. Diral E, Mori S, Antolini L, et al. Increased tumor burden in patients with chronic myeloid leukemia after 36 months of imatinib discontinuation. *Blood.* 2020;136(19):2237–2240. doi:10.1182/blood.2019004371
112. Kimura S, Imagawa J, Murai K, et al. Treatment-free remission after first-line dasatinib discontinuation in patients with chronic myeloid leukaemia (first-line DADI trial): a single-arm, multicentre, phase 2 trial. *Lancet Haematol.* 2020;7(3):e218–25. doi:10.1016/S2352-3026(19)30235-2
113. Fava C, Rege-Cambrin G, Dogliotti I, et al. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. *Haematologica.* 2019;104(8):1589–1596. doi:10.3324/haematol.2018.205054

114. Pagnano KB, Seguro FS, Miranda EC, et al. Duration of major molecular response and discontinuation in deep molecular response (MR4.5) were associated with longer treatment-free survival after imatinib discontinuation - results from two prospective Brazilian trials. *Blood*. 2019;134:1655. doi:10.1182/blood-2019-125368
115. Shah NP, García-Gutiérrez V, Jiménez-Velasco A, et al. Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. *Leuk Lymphoma*. 2020;61(3):650–659. doi:10.1080/10428194.2019.1675879
116. Nagafuji K, Matsumura I, Shimose T, et al. Cessation of nilotinib in patients with chronic myelogenous leukemia who have maintained deep molecular responses for 2 years: a multicenter phase 2 trial, stop nilotinib (NILSt). *Int J Hematol*. 2019;110(6):675–682. doi:10.1007/s12185-019-02736-5
117. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *Lancet Oncol*. 2018;19(6):747–757. doi:10.1016/S1473-2045(18)30192-X
118. Takahashi N, Tauchi T, Kitamura K, et al. Deeper molecular response is a predictive factor for treatment-free remission after imatinib discontinuation in patients with chronic phase chronic myeloid leukemia: the JALSG-STIM213 study. *Int J Hematol*. 2018;107(2):185–193. doi:10.1007/s12185-017-2334-x
119. Kumagai T, Nakaseko C, Nishiwaki K, et al. Dasatinib cessation after deep molecular response exceeding 2 years and natural killer cell transition during dasatinib consolidation. *Cancer Sci*. 2018;109(1):182–192. doi:10.1111/cas.13430
120. Takahashi N, Nishiwaki K, Nakaseko C, et al. Treatment-free remission after two-year consolidation therapy with nilotinib in patients with chronic myeloid leukemia: STAT2 trial in Japan. *Haematologica*. 2018;103(11):1835–1842. doi:10.3324/haematol.2018.194894
121. Fujisawa S, Ueda Y, Usuki K, et al. Feasibility of the imatinib stop study in the Japanese clinical setting: delightedly overcome CML expert stop TKI trial (DOMEST Trial). *Int J Clin Oncol*. 2019;24(4):445–453. doi:10.1007/s10147-018-1368-2
122. Hernández-Boluda JC, Pereira A, Pastor-Galán I, et al. Feasibility of treatment discontinuation in chronic myeloid leukemia in clinical practice: results from a nationwide series of 236 patients. *Blood Cancer J*. 2018;8(10):91. doi:10.1038/s41408-018-0125-0
123. Mahon FX, Boquimpani C, Kim DW, et al. Treatment-free remission after second-line nilotinib treatment in patients with chronic myeloid leukemia in chronic phase: results from a single-group, phase 2, open-label study. *Ann Intern Med*. 2018;168(7):461–470. doi:10.7326/M17-1094
124. Hochhaus A, Masszi T, Giles FJ, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. *Leukemia*. 2017;31(7):1525–1531. doi:10.1038/leu.2017.63
125. Rea D, Nicolini FE, Tulliez M, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: interim analysis of the STOP 2G-TKI study. *Blood*. 2017;129(7):846–854. doi:10.1182/blood-2016-09-742205
126. Kim DDH, Bence-Bruckler I, Forrest DL, et al. Treatment-Free Remission Accomplished By Dasatinib (TRAD): preliminary results of the pan-Canadian tyrosine kinase inhibitor discontinuation trial. *Blood*. 2016;128(22):1922. doi:10.1182/blood.V128.22.1922.1922
127. Imagawa J, Tanaka H, Okada M, et al. Discontinuation of dasatinib in patients with chronic myeloid leukaemia who have maintained deep molecular response for longer than 1 year (DADI trial): a multicentre phase 2 trial. *Lancet Haematol*. 2015;2(12):e528–535. doi:10.1016/S2352-3026(15)00196-9
128. Ross DM, Branford S, Seymour JF, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood*. 2013;122(4):515–522. doi:10.1182/blood-2013-02-483750
129. Rousselot P, Charbonnier A, Cony-Makhoul P, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. *J Clin Oncol off J Am Soc Clin Oncol*. 2014;32(5):424–430. doi:10.1200/JCO.2012.48.5797
130. Thielen N, van der Holt B, Cornelissen JJ, et al. Imatinib discontinuation in chronic phase myeloid leukaemia patients in sustained complete molecular response: a randomised trial of the Dutch-Belgian cooperative trial for haemato-oncology (HOVON). *Eur J Cancer Oxf Engl*. 2013;49(15):3242–3246.
131. Mahon FX, Nicolini FE, Noël MP, et al. Preliminary report of the STIM2 study: a multicenter stop imatinib trial for chronic phase chronic myeloid leukemia de novo patients on imatinib. *Blood*. 2013;122(21):654. doi:10.1182/blood.V122.21.654.654
132. Yhim HY, Lee NR, Song EK, et al. Imatinib mesylate discontinuation in patients with chronic myeloid leukemia who have received front-line imatinib mesylate therapy and achieved complete molecular response. *Leuk Res*. 2012;36(6):689–693. doi:10.1016/j.leukres.2012.02.011
133. Matsuki E, Ono Y, Tonegawa K, et al. Detailed Investigation on characteristics of Japanese patients with chronic phase CML who achieved a durable CMR after discontinuation of imatinib – an updated result of the keio STIM study. *Blood*. 2012;120(21):2788. doi:10.1182/blood.V120.21.2788.2788
134. Vigón L, Luna A, Galán M, et al. Identification of immunological parameters as predictive biomarkers of relapse in patients with chronic myeloid leukemia on treatment-free remission. *J Clin Med*. 2021;10(1):42. doi:10.3390/jcm10010042
135. Rea D, Henry G, Khaznadar Z, et al. Natural killer-cell counts are associated with molecular relapse-free survival after imatinib discontinuation in chronic myeloid leukemia: the IMMUNOSTIM study. *Haematologica*. 2017;102(8):1368–1377. doi:10.3324/haematol.2017.165001
136. Raspadori D, Pacelli P, Sicuranza A, et al. Flow cytometry assessment of CD26+ leukemic stem cells in peripheral blood: a simple and rapid new diagnostic tool for chronic myeloid leukemia. *Cytometry B Clin Cytom*. 2019;96(4):294–299. doi:10.1002/cyto.b.21764
137. Bocchia M, Sicuranza A, Abruzzese E, et al. Residual peripheral blood CD26+ leukemic stem cells in chronic myeloid leukemia patients during TKI Therapy and during treatment-free remission. *Front Oncol*. 2018;8:194. doi:10.3389/fonc.2018.00194
138. Stuckey R, López Rodríguez JF, Gómez-Casares MT. Discontinuation of tyrosine kinase inhibitors in patients with chronic myeloid leukemia: a review of the biological factors associated with treatment-free remission. *Curr Oncol Rep*. 2022;24(4):415–426. doi:10.1007/s11912-022-01228-w
139. Cayssials E, Jacomet F, Piccirilli N, et al. Sustained treatment-free remission in chronic myeloid leukaemia is associated with an increased frequency of innate CD8(+) T-cells. *Br J Haematol*. 2019;186(1):54–59. doi:10.1111/bjh.15858
140. Claudiani S, Apperley JF, Khan A, Khorashad J, Milojkovic D. Prolonged treatment-free remission in chronic myeloid leukemia patients with previous BCR-ABL1 kinase domain mutations. *Haematologica*. 2020;105(5):e225–7. doi:10.3324/haematol.2019.234179
141. D'Adda M, Farina M, Schieppati F, et al. The e13a2 BCR-ABL transcript negatively affects sustained deep molecular response and the achievement of treatment-free remission in patients with chronic myeloid leukemia who receive tyrosine kinase inhibitors. *Cancer*. 2019;125(10):1674–1682. doi:10.1002/cncr.31977

142. Castagnetti F, Binotto G, Capodanno I, et al. Making Treatment-Free Remission (TFR) easier in chronic myeloid leukemia: fact-checking and practical management tools. *Target Oncol.* **2021**;16(6):823–838. doi:10.1007/s11523-021-00831-4
143. Mori S, Vagge E, le Coutre P, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. *Am J Hematol.* **2015**;90(10):910–914. doi:10.1002/ajh.24120
144. Atallah E, Schiffer CA, Radich JP, et al. Assessment of outcomes after stopping tyrosine kinase inhibitors among patients with chronic myeloid leukemia: a nonrandomized clinical trial. *JAMA Oncol.* **2021**;7(1):42–50. doi:10.1001/jamaoncol.2020.5774
145. Clark RE, Polydoros F, Apperley JF, et al. De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. *Lancet Haematol.* **2019**;6(7):e375–83. doi:10.1016/S2352-3026(19)30094-8
146. Atallah E, Treatment-Free Remission: SK. the New Goal in CML Therapy. *Curr Hematol Malig Rep.* **2021**;16(5):433–439. doi:10.1007/s11899-021-00653-1
147. Kim DDH, Novitzky-Basso I, Kim TS, et al. Optimal duration of imatinib treatment/deep molecular response for treatment-free remission after imatinib discontinuation from a Canadian tyrosine kinase inhibitor discontinuation trial. *Br J Haematol.* **2021**;193(4):779–791. doi:10.1111/bjh.17447
148. ECOG-ACRIN Cancer Research Group. Phase II study of adding the anti-PD-1 pembrolizumab to tyrosine kinase inhibitors in patients with chronic myeloid leukemia and persistently detectable minimal residual disease; **2021**. Available from: <https://clinicaltrials.gov/ct2/show/NCT03516279>. Accessed February 10, 2023.
149. Katagiri S, Tauchi T, Ando K, Okabe S, Gotoh M, Ohyashiki K. Low body weight and body mass index may be associated with musculoskeletal pain following imatinib discontinuation in chronic myeloid leukemia. *Leuk Res Rep.* **2017**;7:33–35. doi:10.1016/j.lrr.2017.04.002
150. Richter J, Söderlund S, Lübking A, et al. Musculoskeletal Pain in Patients With Chronic Myeloid Leukemia After Discontinuation of Imatinib: a Tyrosine Kinase Inhibitor Withdrawal Syndrome? *J Clin Oncol.* **2014**;32(25):2821–2823. doi:10.1200/JCO.2014.55.6910
151. Flynn KE, Atallah E, Lin L, et al. Patient- and physician-reported pain after tyrosine kinase inhibitor discontinuation among patients with chronic myeloid leukemia. *Haematologica.* **2022**;107(11):2641–2649. doi:10.3324/haematol.2021.280377

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Managing the Cerebrovascular Complications of Sickle Cell Disease: Current Perspectives

Jennifer Light, Maria Boucher, Jacquelyn Baskin-Miller & Mike Winstead

To cite this article: Jennifer Light, Maria Boucher, Jacquelyn Baskin-Miller & Mike Winstead (2023) Managing the Cerebrovascular Complications of Sickle Cell Disease: Current Perspectives, Journal of Blood Medicine, , 279-293, DOI: [10.2147/JBM.S383472](https://doi.org/10.2147/JBM.S383472)

To link to this article: <https://doi.org/10.2147/JBM.S383472>



© 2023 Light et al.



Published online: 14 Apr 2023.



Submit your article to this journal



Article views: 500



View related articles



View Crossmark data



Citing articles: 9 View citing articles

Managing the Cerebrovascular Complications of Sickle Cell Disease: Current Perspectives

Jennifer Light*, Maria Boucher*, Jacquelyn Baskin-Miller*, Mike Winstead*

Pediatric Hematology-Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

*These authors contributed equally to this work

Correspondence: Mike Winstead, Division of Pediatric Hematology-Oncology, University of North Carolina at Chapel Hill, 101 Manning Drive, Chapel Hill, NC, USA, Tel +1 919-966-1178, Fax +1 919-966-7629, Email mwins@email.unc.edu

Abstract: The importance of protecting brain function for people with sickle cell disease (SCD) cannot be overstated. SCD is associated with multiple cerebrovascular complications that threaten neurocognitive function and life. Without screening and preventive management, 11% of children at 24% of adults with SCD have ischemic or hemorrhagic strokes. Stroke screening in children with SCD is well-established using transcranial Doppler ultrasound (TCD). TCD velocities above 200 cm/s significantly increase the risk of stroke, which can be prevented using chronic red blood cell (RBC) transfusion. RBC transfusion is also the cornerstone of acute stroke management and secondary stroke prevention. Chronic transfusion requires long-term management of complications like iron overload. Hydroxyurea can replace chronic transfusions for primary stroke prevention in a select group of patients or in populations where chronic transfusions are not feasible. Silent cerebral infarction (SCI) is even more common than stroke, affecting 39% of children and more than 50% of adults with SCD; management of SCI is individualized and includes careful neurocognitive evaluation. Hematopoietic stem cell transplant prevents cerebrovascular complications, despite the short- and long-term risks. Newer disease-modifying agents like voxelotor and crizanlizumab, as well as gene therapy, may treat cerebrovascular complications, but these approaches are investigational.

Keywords: stroke, silent cerebral infarction, moyamoya syndrome, transfusion, stem cell transplantation

Plain Language Summary

Sickle cell disease (SCD) is an inherited disease that affects the red blood cells (RBCs). A genetic mutation causes the RBCs to change from a flat, round shape into a long, curved “sickle” shape. Sickled RBCs disrupt blood flow and cause injury to the inner lining of the blood cells. Disrupting blood flow to the brain can lead to a stroke, where areas of the brain stop functioning. Strokes can be fatal or cause permanent difficulty speaking, moving, or learning. SCD can also cause silent infarctions or “silent strokes”, which actually cause symptoms like difficulty learning. Previously, one-quarter of people with SCD had a stroke by age 45, and many of these strokes occurred in children. Today, strokes in children with SCD can be prevented by checking a transcranial Doppler (TCD) ultrasound every year and providing blood transfusions to children with abnormal blood flow on the TCD. If a person with SCD has a stroke, they need an emergency exchange transfusion to remove the “sickled” blood. Preventing stroke requires monthly blood transfusions for life, but there is interest in other treatments. For certain people with SCD, the medication hydroxyurea can prevent stroke. Stem cell or bone marrow transplantation can cure SCD but may not be possible for everyone. Researchers are studying newer medications and gene therapy, but the safety and effectiveness of these treatments are not yet known. Healthcare disparities also make it harder for many people with SCD to get treatment.

Introduction

Cerebrovascular complications of sickle cell disease (SCD) result from widespread vasculopathy. SCD is an autosomal recessive condition caused by a point mutation in the *HBB* gene, which causes a single amino acid substitution in the β -globin chain.¹⁻⁴ The resulting hemoglobin S (HbS) variant polymerizes in its deoxygenated state, which causes the characteristic “sickle” shape change in erythrocytes when exposed to hypoxia.⁵ Sickled

erythrocytes interfere with the normal rheology of the microvascular circulation,⁶ leading to vaso-occlusion and reperfusion injury.⁷ Erythrocytes containing HbS also develop premature senescence⁸ and have a shortened lifespan in the circulation, resulting in chronic intra- and extravascular hemolysis.⁹ Other factors like sleep disordered breathing may contribute to intermittent erythrocyte sickling and tissue hypoxia.^{10,11}

Ischemia-reperfusion injury and hemolysis contribute to an inflammatory milieu^{12,13} and endothelial activation.¹⁴ Hypercoagulability results from complex interactions between the coagulation system and the endothelium, including von Willebrand factor release, nitric oxide depletion, upregulation of vascular adhesion molecules like P-selectin,¹⁵ interactions with microparticles, and alterations in the activity of intrinsic anticoagulants like protein C and protein S.^{16–19} Chronic vasculitis and hypercoagulability in the carotid, vertebral, and cerebral arteries lead to acute infarctive and ischemic stroke, as well as chronic complications like carotid stenosis and moyamoya syndrome.²⁰

Stroke and other complications of cerebrovascular disease represent significant sources of morbidity and mortality for people with SCD. In the longitudinal Cooperative Study of SCD, the estimated prevalence of stroke was 11% by age 19 years and 24% by age 45 years, with the highest risk and lowest age at first stroke among people with homozygous hemoglobin S (HbSS) disease and hemoglobin S β^0 (HbS β^0) thalassemia.²¹ Stroke risk is lower among people with hemoglobin SC (HbSC) disease^{21,22} Hemoglobin S β^+ (HbS β^+) thalassemia and other compound heterozygous forms of SCD have variable stroke risk, which may be related to degree of anemia and hemolysis.²³

Strokes in people with SCD frequently involve the distributions of median cerebral arteries, due to chronic narrowing of the internal carotid arteries, but strokes can also involve the vertebrobasilar arteries and other vessels along the Circle of Willis, potentially in association with vascular anomalies like moyamoya syndrome.^{24–26} Neurocognitive and psychological complications of cerebrovascular disease affect children and adults with SCD,^{27–30} requiring patients, families, and providers to be aware of screening practices and interventions to prevent life-threatening complications and improve quality of life.

Primary Stroke Prevention

Importance of Transcranial Doppler Screening

Primary stroke prevention identifies patients without a known prior stroke that are at high risk for an event. For people with SCD, the cornerstone of primary stroke prevention is routine screening with transcranial Doppler ultrasound (TCD). A landmark series of 283 TCDs in 190 patients with SCD found that stroke correlated with increasing blood-flow velocity in the median cerebral arteries, a marker of vascular narrowing; strokes occurred in 7 of 23 participants with elevated velocity, compared to none with normal velocity.²⁴ Identifying individuals at high risk allows effective preventive treatment with chronic transfusion therapy. The availability of effective intervention, as well as strong correlation between TCD and more complex studies like cerebral angiography and magnetic resonance imaging (MRI) with magnetic resonance angiography (MRA),^{31–33} reinforce the utility, reliability, and importance of TCD as a screening technique.

For children with SCD, screening with TCD should be performed annually in patients aged 2 to 16 years with HbSS or HbS β^0 thalassemia.²³ TCD is not currently recommended for children with HbSC.²³ For children with other forms of heterozygous SCD like HbS β^+ thalassemia, TCD may be indicated if the clinical and laboratory phenotype resembles homozygous SCD.²³ TCD measures the time-averaged mean maximum velocity (TAMMV) of blood flow in the bilateral median cerebral arteries; velocities are generally interpreted as normal, conditional, or abnormal. A TAMMV ≥ 200 cm/s is associated with a 40% increased risk of stroke within 3 years and is the recommended cut-off for abnormal.^{23,34} Velocities in the conditional range of 170–199 cm/s are at significant risk of rising into the high-risk range³⁵ and should be followed closely. Our usual practice is to repeat TCD within 3 months for velocities of 170–184 cm/s and within 1 month for velocities of 185–199 cm/s.

These values were established by non-imaging TCD. Imaging TCD uses different technical methods and provides slightly different estimates of velocity.^{36,37} Current guidelines recommend adjusting the threshold for chronic transfusion based on institutional TCD methods; for imaging TCD, high risk of stroke is indicated by two measurements ≥ 185 cm/s or any measurement ≥ 205 cm/s.²³

Use of TCD may be limited by difficulty in obtaining adequate sonographic windows, inter-operator variability, and inability to obtain accurate readings in the vertebrobasilar arteries.^{24,31} For children in whom adequate TCD readings cannot be obtained, despite multiple attempts by experienced operators, our practice is to obtain brain MRI and MRA. In young children these studies usually require sedation, so the treatment team must weigh the risks and benefits collaboratively with the patient's caregivers; pre-anesthesia transfusion is indicated. Healthcare disparities can also affect the availability of comprehensive care for people with SCD.³⁸ Providers must be aware of societal and racial inequities that affect healthcare for people with SCD.³⁹ At present, most strokes in children with SCD in the United States are associated with inadequate TCD screening or delayed initiation of chronic transfusion, gaps in care influenced by systemic inequity in healthcare resources.²⁵

Even in those with no history of abnormal TCDs, screening MRI may identify silent infarctions. Based on current guidelines, a screening MRI of the brain is appropriate for asymptomatic adults with SCD and for asymptomatic children with SCD who are old enough to undergo MRI without sedation,²³ typically around age 10 years in our practice. It is also important to evaluate children for other treatable comorbidities like sleep disordered breathing, which is likely underdiagnosed and may be a risk factor for stroke.^{10,11}

Initiation of Chronic Transfusions

For patients with elevated stroke risk, the standard of care is early initiation of chronic transfusion therapy. The landmark Stroke Prevention Trial in Sickle Cell Anemia (STOP) was a randomized controlled trial that showed the effectiveness of this management strategy. The benefit of chronic transfusion was pronounced, with a 93% reduction in the relative risk of stroke in the transfused group; the trial ended early due to clear evidence of benefit.^{40,41} Chronic transfusions must be started in collaboration with the patient and family, including a clear discussion of the risks and benefits. Switching from chronic transfusion to hydroxyurea may be feasible for some patients (see “Duration of Transfusion Therapy and the Role of Hydroxyurea” below). For many patients, however, chronic transfusions are a lifelong intervention,⁴² requiring long-term management of complications like iron overload.

The usual approach to therapy is red blood cell (RBC) transfusion every 3 to 4 weeks, maintaining a hemoglobin concentration of at least 9 g/dl and a fraction of HbS <30% between transfusions.²³ This goal is based on the methodologies of STOP and its successor study, STOP2, as well as animal models and blood viscosity studies.^{40,42–45} It is not necessary to maintain a normal hemoglobin concentration, as people with SCD adapt to chronic anemia and can be vulnerable to increased blood viscosity; our practice is to maintain a post-transfusion hemoglobin concentration of 10 to 11 g/dl.

Patients receiving chronic transfusion therapy may benefit from exchange transfusion, rather than simple transfusion (Table 1).^{46,47} In exchange transfusion, a predetermined volume of blood is removed while RBCs are being transfused, either by apheresis (automated exchange) or by a medical provider (manual exchange).⁴⁷ Exchange transfusion provides greater control over the end-transfusion hemoglobin concentration and HbS and can also decrease iron overload, although patients receiving exchange transfusion still require long-term monitoring of iron status. Exchange transfusion is more resource-intensive than simple transfusion. Manual exchange (see “Management of Acute Stroke” below) requires one-to-one care for the duration of the procedure. Automated exchange requires an apheresis device and trained transfusion-medicine staff; priming the apheresis machine requires additional transfusion volume, and the procedure is generally not indicated for small children. Venous access for automated exchange transfusion may require an apheresis-compatible central venous access device; infection risk, MRI compatibility of the access device, and education for patients and caregivers are important in this context.

Transfusion Complications and Iron Overload

Chronic transfusion can be life-saving for people with SCD at high risk of stroke, but there are risks, particularly transfusion reactions and iron overload.⁴⁶ Immune-mediated transfusion reactions include acute and delayed hemolytic reactions, as well as alloimmunization. In people with SCD, severe delayed hemolytic transfusion reactions can involve reticulocytopenia and “bystander” hemolysis, leading to profound anemia and a risk of cerebral ischemia.^{48,49} Close

Table I Approaches to Chronic Transfusion Therapy

Method	Simple Transfusion	Manual Exchange Transfusion	Automated Exchange Transfusion
Goal Hb	10–11 g/dl		
Goal HbS	<30%		
Procedure	Intravenous infusion of 10–15 mL/kg RBCs	Phlebotomize whole blood and transfuse aliquots of RBCs	Remove and infuse RBCs via apheresis
Access required	1 PIV (any size)	1–2 PIVs (moderate size for phlebotomy)	2 PIVs (large) or CVAD
Post-transfusion Hb	Always higher than pre-transfusion	Higher or equal to pre-transfusion	Better control than manual exchange
Likelihood of reaching goal HbS	Moderate	Moderately high	High
Iron loading	Rapid	Moderate	Slow
Time	2–3 hours	3–4 hours	1–2 hours
Availability	Widespread	Widespread	Limited
Cost	Low	Moderate	High

Note: CVAD for automated exchange must be apheresis-compatible and should be MRI-compatible.

Abbreviations: Hb, hemoglobin concentration; HbS, hemoglobin S fraction; RBCs, transfused red blood cells; PIV, peripheral intravenous catheter; CVAD, central venous access device.

collaboration between the patient, the hematology team, and the transfusion medicine service is essential for prompt recognition and management of transfusion reactions, as well as prevention.

Individuals with SCD receiving chronic transfusions are at high risk of alloantibody formation, due to repeated exposure to minor antigens on donor RBCs. Alloimmunization can occur to any minor RBC antigen, and it is essential that a person with a history of alloimmunization not be exposed to the mismatched antigen again. The antiglobulin tests of alloimmunized individuals may normalize over time, so it is essential that hematology and transfusion medicine carefully track transfusion reactions.

In addition to standard matching of the ABO antigens and the Rhesus-D antigen, people receiving chronic transfusions require more extensive matching of minor antigens. The most immunogenic antigens for people with SCD are in Rhesus group (C, c, E, e) and the Kell group (K).⁵⁰ All RBC transfusions for people with SCD should be matched at C, E, and K.^{46,51} More extended matching of minor RBC blood groups like Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), and MNSs may further reduce the risk of alloimmunization, but this practice limits the pool of blood donors, and the overall benefit is unclear.^{44,46,52} For recently-transfused patients, molecular studies of the peripheral blood are not accurate indicators of the patient's RBC phenotype. In this situation, genetic studies are an alternative. There can be discrepancies between genotype and RBC phenotype.⁵³

Iron overload is a ubiquitous complication of chronic transfusions, including exchange transfusion. Because the body does not have a physiological mechanism for eliminating large amounts of iron, the iron from transfused RBCs accumulates in the endocrine organs, liver, and myocardium, which can lead to life-limiting organ dysfunction.^{54,55} Serum ferritin levels increase with iron burden^{55,56} and should be monitored at least quarterly in patients receiving chronic transfusions. Ferritin levels alone, however, do not predict liver iron concentration (LIC) or other sequelae of iron overload.⁵⁶ Additional monitoring of iron burden should include MRI of the liver and of the heart with T2* sequences (which have generally replaced liver biopsy⁵⁷) every 1 to 2 years, particularly in those with a ferritin concentration >1000 µg/L.⁴⁶ Chelation therapy is generally indicated for patients with evidence of cardiac iron loading (T2* <20 ms), LIC of at least 3.5 mg/g, or serum ferritin consistently above 1000 µg/L, although other thresholds may be considered.^{54,55}

Chelation therapy must be individualized and carefully monitored for efficacy and tolerability. Deferoxamine is highly effective but must be given either intravenously or as a daily subcutaneous infusion, which makes it challenging for home use. Deferasirox is an oral chelating agent that has similar efficacy to deferoxamine and is often more feasible for chronic outpatient use.^{58,59} Personal, social, and economic factors may interfere with a person's ability to take their chelating agent as prescribed, and careful follow-up is needed to ensure adherence.⁶⁰ Patients taking deferoxamine or deferasirox should be monitored for ocular, hepatic, or renal toxicity, and there is a small risk of opportunistic infection.^{61,62} Deferiprone, another effective oral chelating agent, has been studied more recently in people with SCD, although its association with neutropenia may limit its use.^{63,64}

Duration of Transfusion Therapy and the Role of Hydroxyurea

For people at high risk of stroke based on childhood TCD, primary stroke prophylaxis is a lifelong undertaking. In the STOP2 trial, stopping transfusion (without other prophylaxis) significantly increased the risks of rising TCD velocity and overt stroke.⁴² More recently, the TCD with Transfusions Changing to Hydroxyurea (TWiTCH) trial found that hydroxyurea (hydroxycarbamide) can effectively stabilize TCD velocities in carefully selected patients (Table 2).⁶⁵ Participants in TWiTCH did not have a history of stroke, TIA, or severe vasculopathy (defined as carotid stenosis on MRA). All participants received chronic transfusion therapy for at least 12 months, and transfusions overlapped with hydroxyurea until reaching the maximum tolerated hydroxyurea dose. Although the TWiTCH trial included participants who had abnormal TCDs while receiving chronic transfusions, our practice is to only consider changing to hydroxyurea in patients whose TCDs have normalized.

When considering the transition from chronic transfusions to hydroxyurea, the care team must be cautious and partner with the patient and other caregivers. Like transfusion, primary stroke prevention with hydroxyurea must be continued lifelong. Because hydroxyurea is given at home, it is essential that the patient and family understand the need for strict adherence and also that the care team is aware of social factors that may complicate the patient's ability to obtain and

Table 2 Inclusions and Exclusions for Changing Primary Stroke Prevention from Chronic Transfusion to hydroxyurea⁶⁵

Inclusion Criteria	
Diagnosis	Sickle cell anemia
Age	4 to 16 years
Transfusion duration	≥12 months
TCD velocity on chronic transfusion	Normal or abnormal
Required studies	Brain MRI and MRA
Exclusion Criteria	
Clinical history	Stroke
	Transient ischemic attack
	Prior severe vasculopathy
MRA findings	Moderate stenosis in 2 or more arteries
	Any severe arterial stenosis
TCD	Inadequate windows

Note: Our practice is to continue chronic transfusion therapy in patients with persistently abnormal TCDs.

Abbreviations: TCD, transcranial Doppler ultrasound; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography.

take their medication.^{66–68} Continued TCD monitoring is necessary. Hydroxyurea appears to be safe for long-term use, but study of its secondary effects, such as infertility, is ongoing.^{69–71}

In the randomized, controlled Pediatric Hydroxyurea Phase 3 Clinical Trial (BABY HUG) trial, starting hydroxyurea in early childhood was associated with a slower rise in TCD, compared to placebo.^{72,73} An ongoing clinical trial is evaluating the up-front use of hydroxyurea for primary stroke prophylaxis in regions without access to chronic transfusion.⁷⁴

Silent Cerebral Infarction

Silent cerebral infarction (SCI) is an abnormality on MRI that does not correlate with a permanent, focal neurological deficit.^{75–77} Based on observational studies with surveillance MRI, the prevalence of SCI rises with age; around 39% of children and SCD and more than 50% of adults with SCD have silent infarctions.^{23,76} Despite its name, SCI does manifest clinically as neurocognitive decline, difficulty with school and work performance, and other neuropsychological problems.^{27,76} SCI is also a significant risk factor for overt stroke.⁷⁵ Recent reports suggest that SCI in people with HbSC disease may be more common than previously recognized.^{78,79}

Once SCI is identified, it requires ongoing radiographic and clinical evaluation. Management of SCI is an individual process. The Silent Cerebral Infarct Multicenter Clinical Trial (SIT) showed that chronic transfusion therapy in children with SCI reduced the risks of overt stroke and extension of SCI, with 6% of transfused participants and 14% of control participants developing one of these outcomes.⁷⁷ Given the relatively high number needed to treat and the significant transfusion burden, the decision to initiate chronic transfusions for SCI must be individualized.²³ For children with SCI and significant schooling difficulty, for example, the benefits of chronic transfusion may outweigh the risks, although there is no standardized practice. Hydroxyurea may help prevent development and progression of SCI, although evidence is limited.^{80–82}

Acute Stroke

Diagnosis

Because individuals with sickle cell disease (especially HbSS and HbS β^0 thalassemia) are at high risk of stroke, it is important to have a high index of suspicion of stroke in this population. Clinicians should suspect a stroke or transient ischemic attack (TIA) in any patient with an acute neurological deficits, such as hemiparesis, ataxia, or dysphasia; altered consciousness, including new-onset seizures; or acute headache.^{83–85} While an emergent, non-contrast-enhanced CT scan of the brain is necessary to evaluate for intracranial hemorrhage, the best evaluation of stroke is MRI with MRA, which can delineate the affected areas and also distinguish other conditions that mimic stroke.⁸⁶ Because strokes can arise from the vertebrobasilar circulation, MRA must include the vertebral arteries. Treatment should be initiated within 2 hours of symptom onset.²³ If emergent MRI and MRA are not available, treatment should not be delayed.

Management of Acute Stroke

Due to the pathophysiology of stroke in sickle cell disease, the treatment differs from stroke in other patients. The main goal is to decrease the fraction of HbS to less than 30% using emergent RBC transfusion. Ideally, this is done through an emergency exchange transfusion, which decreases the HbS fraction more efficiently than a simple transfusion and is associated with a five-fold lower risk of secondary stroke.^{46,87,88} Emergent exchange transfusion may be complicated by the location and resources of the initial treating facility, as well as vascular access and patient acuity. Rather than delaying treatment, it may be necessary to perform an initial simple transfusion using C, E, and Kell-matched blood, targeting a hemoglobin concentration of 10–11 g/dL, followed by exchange transfusion as soon as possible.^{43,46,86} Other medical therapies for stroke like tissue plasminogen activator are controversial in patients with SCD²³ and are not substitutes for transfusion.

Any patient with SCD and an acute stroke should be transferred to an intensive care unit equipped to manage neurological disorders as safely and quickly as possible. Exchange transfusion for acute stroke is best performed by automated apheresis.⁴⁶ Depending on local resources, however, manual exchange may be more feasible to avoid delays.

For a manual exchange, the volumes of blood to remove and transfuse can be calculated using the patient's body weight, starting hemoglobin/hematocrit, and starting HbS percentage (assume 100% if unavailable).

Our approach to manual exchange (Table 3) is to estimate the patient's total blood volume (Step 1) and then calculate the desired change in HbS percentage (Step 2) to reach 30% or less. Multiplying these values (Step 3) gives the volume of whole blood to remove. Because transfused RBCs are more concentrated than RBCs in vivo, the erythrocyte volume of the whole blood must be calculated, based on the patient's hematocrit (Step 4); this erythrocyte volume can then be converted to an equivalent transfusion volume using the hematocrit of transfused RBCs, typically around 60% (Step 5). To raise the final hemoglobin concentration requires adding approximately 5 mL/kg of transfused RBCs for every 1 g/dl change in hemoglobin concentration desired (Steps 6, 7). To prevent fluid shifts and cardiopulmonary decompensation, boluses of crystalloid are given before and after exchange, and blood is removed in aliquots over several hours, alternating with transfusion.

For acute ischemic stroke in patients with SCD, there is little role for surgical intervention. Neurosurgical or endovascular intervention may rarely be indicated for intracranial hemorrhage.⁸⁹ For patients with critical carotid stenosis or moyamoya syndrome, revascularization procedures may be indicated after recovery from the acute stroke (see "Surgical Revascularization" below).

Other Cerebrovascular Complications Presenting Similarly to Stroke

While emergent management of stroke is appropriate for patients with SCD and acute neurological findings, other disorders can present similarly. Recently, it has been reported that people with SCD are at increased risk of posterior reversible encephalopathy syndrome (PRES).⁹⁰ PRES involves altered mental status, headache, vision changes, and a characteristic finding on MRI of posterior cerebral white matter edema.⁹¹ Risk factors for PRES are overrepresented

Table 3 Sample Calculations for Manual Exchange Transfusion

Phlebotomy and transfusions volumes		Example: 30-kg child with HbS 90%, Hb 7 g/dl, HCT 21%
1. Estimate the patient's total blood volume (TBV). $TBV = \text{Weight (kg)} \times 75 \frac{\text{ml}}{\text{kg}}$	1. ____ ml	$30 \text{ kg} \times 75 \frac{\text{ml}}{\text{kg}} = 2250 \text{ ml}$
2. Calculate the desired change in HbS (ΔHbS). $\Delta\text{HbS} = 1 - \left(\frac{\text{Goal HbS (\%)}}{\text{Starting HbS (\%)}} \right)$	2. ____	$1 - \left(\frac{30\%}{90\%} \right) = 0.67$
3. Calculate the whole-blood volume to remove (WBV). $WBV = TBV \times \Delta\text{HbS}$	3. ____ ml	$2250 \text{ ml} \times 0.67 = 1500 \text{ ml}$
Infuse 10–20 ml/kg of crystalloid before starting. Remove this volume in 4–6 aliquots, 15–30 minutes apart.		Infuse 300–600 ml crystalloid. Remove 300 ml every 30 minutes for 5 cycles.
4. Calculate the erythrocyte volume to remove (EV). $EV = \frac{WBV \times \text{Patient HCT}}{100}$	4. ____ ml	$\frac{1500 \text{ ml} \times 21}{100} = 315 \text{ ml}$
5. Convert the EV to a base RBC volume to transfuse. $\text{Base RBC volume} = \frac{EV \times 100}{\text{HCT of transfused RBCs}}$	5. ____ ml	$\frac{315 \text{ ml} \times 100}{60} = 525 \text{ ml}$
6. Add RBC volume to raise the hemoglobin concentration. $\text{Added RBC volume} = \text{Weight (kg)} \times (\text{Goal Hb} - \text{Starting Hb}) \times 5$	6. ____ ml	$30 \text{ kg} \times (10 - 7) \times 5 = 450 \text{ ml}$
7. Calculate the total RBC volume to transfuse. $\text{Total RBC volume} = \text{Base volume} + \text{Added volume}$	7. ____ ml	$525 \text{ ml} + 450 \text{ ml} = 975 \text{ ml}$
Transfuse in 4–6 aliquots, between phlebotomy cycles. Infuse 10–20 ml/kg of crystalloid after completion.		Transfuse 195 ml every 30 minutes for 5 cycles. Infuse 300–600 ml crystalloid.

Notes: There are multiple equations to estimate TBV. Assume a starting HbS of 100% if the patient's HbS is not available. Hematocrit of RBC units and the expected rise in Hb per mL transfused may vary by institution; consult local blood bank.

Abbreviations: HbS, hemoglobin S; HCT, hematocrit; Hb, hemoglobin concentration (g/dl); RBC, transfused red blood cells.

among people with SCD, including hypertension, renal disease, immunosuppressant therapy, and blood transfusion,^{92,93} Sickle cell disease is a hypercoagulable state,¹⁹ with protein C and protein S dysregulation, and individuals with SCD may also be at an increased risk of cerebral venous sinus thrombosis or thrombotic complications of hospitalization.^{94,95}

Stroke Aftercare

Secondary Stroke Prevention

Secondary prophylaxis refers to the prevention of subsequent strokes in patients with a history of stroke. Without preventive treatment after a first stroke, 67% of people with SCD in one series had an additional stroke.⁸³ The earliest reports of chronic transfusion therapy in SCD were for secondary stroke prevention in the 1970s,⁹⁶ an approach supported by subsequent studies.^{97,98} Due to the lack of equipoise and the danger of withholding prophylaxis, this practice has not been the subject of controlled trials.⁹⁹ As with primary prevention, chronic transfusion for secondary stroke prevention is generally performed every 3 to 4 weeks, maintaining a hemoglobin >9 g/dl and an HbS <30% between transfusions.²³ Even with chronic transfusions, there is a significant risk of subsequent strokes, as well as other neurocognitive complications.⁹⁸

In contrast to primary prophylaxis, hydroxyurea alone cannot be used for secondary stroke prevention. The Stroke With Transfusions Changing to Hydroxyurea (SWiTCH) trial, a randomized controlled trial of hydroxyurea versus chronic transfusion for secondary stroke prevention, studied stroke risk and iron overload.¹⁰⁰ The trial was closed due to futility, after interim analysis found a 10% prevalence of stroke in the hydroxyurea group, compared to 0% in the transfused group, with no difference in iron burden. Chronic transfusion remains the standard of care for secondary stroke prevention in people with SCD.

Meeting the Needs of Stroke Survivors

Overwhelmingly, the evidence points to a consistent cognitive decline among stroke survivors.³⁰ Routine neurocognitive evaluations are necessary for all patients with a prior stroke,²³ and for children it is important to monitor school performance. Children can succeed in school after strokes, but they may require individualized education plans, and their caregivers may require advocacy and support from the medical team. For young adults with SCD, the transition from pediatric to adult care is associated with a significant risk of worsening disease and death.¹⁰¹ While death rates from SCD have consistently declined among children, they have risen among adults, likely reflecting socioeconomic obstacles to obtaining access to care.¹⁰²

Adult stroke survivors require continuity of high-quality SCD care, including secondary prophylaxis. Cognitive deficits and psychological comorbidities are associated with greater risk during the transition period.^{101,103} Recognizing that the transition to adult care is not a single event but an ongoing process that begins during adolescence and continues through early adulthood,^{104,105} it is essential that stroke survivors, their caregivers, and their providers actively collaborate to ensure continuity of care, regular neurocognitive assessment, continuation of chronic transfusions, and management of iron overload.

Other Therapies to Modify Stroke Risk

Surgical Revascularization

Neurosurgical consultation is indicated for patients with evidence of a severe vasculopathy, including critical arterial stenosis or moyamoya syndrome on MRA. Moyamoya syndrome is a complication of chronic internal carotid artery occlusion, in which friable collateral vessels develop along the base of the brain; cerebral angiography demonstrates the characteristic “wisp of smoke” enhancement in the collateral vessels.¹⁰⁶ Even with chronic transfusion therapy, the rate of stroke and TIA in people with moyamoya was 41% in one series.¹⁰⁷

Indirect revascularization procedures like encephaloduroarteriosynangiosis (EDAS) mobilize blood vessels from the scalp into the calvarium to perfuse areas distal to the stenotic vessels; published experience suggests a low risk of serious surgical complications like hemorrhage, perioperative stroke, or functional impairment.^{23,108,109} As an adjunct to chronic transfusion therapy, surgical intervention may decrease stroke risk, and a recent review of pediatric patients found that

EDAS facilitated return to school.¹¹⁰ However, there are no controlled studies of neurosurgical intervention, and the specific revascularization procedure used typically depends on institution and surgeon preference. Any consideration of neurosurgical intervention requires a multidisciplinary collaboration between hematology, neuroradiology, neurosurgery, and the patient.²³

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) for SCD decreases stroke risk, and a history of stroke is a strong indication to evaluate for HSCT.^{111,112} HSCT replaces the patient's hematopoietic progenitor cells and immune system with those of a related or unrelated donor. Cerebrovascular benefits are established by multiple studies, with improved stenosis scores, improved TCDs, and reduction in strokes post-transplant.^{113,114} Given the availability of effective disease-modifying therapy, however, the risks of HSCT must be carefully considered.^{115–117}

Complications of HSCT include effects of pre-HSCT conditioning therapy, infection, graft rejection, and acute or chronic graft-versus-host disease (GVHD).¹¹⁸ Late effects of conditioning agents like busulfan and cyclophosphamide include infertility and organ dysfunction.¹¹¹ GVHD, which involves tissue destruction by donor lymphocytes, can be life-threatening or interfere considerably with quality of life, especially after unrelated-donor HSCT.¹¹⁹ Research is ongoing to mitigate these risks, including the use of lower-intensity conditioning regimens and enhanced GVHD prophylaxis with agents like abatacept.¹²⁰ Interim analysis from an ongoing clinical trial (ClinicalTrials.gov identifier NCT04018937) of HSCT with reduced-intensity conditioning for young children with SCD and matched sibling donors has reported 100% disease-free survival and no severe GVHD,¹²¹ emphasizing the viability of early HSCT for SCD.

Risks of HSCT are higher among adolescents and adults with SCD, compared to young children. Data from the Sickle Cell Transplant to Prevent Disease Exacerbation (STRIDE) study, however, found that related- or unrelated-donor HSCT is feasible in adults with SCD and severe comorbidities, with 82% event-free survival in this exceptionally high-risk population.¹²² The ongoing STRIDE2 trial (ClinicalTrials.gov identifier NCT02766465) will compare the long-term outcomes of HSCT to standard therapy.¹²³ For adults with matched sibling donors, the National Institutes of Health and other centers successfully piloted HSCT with very low-intensity conditioning, which was associated with 87% to 92% disease-free survival.^{124,125} This approach is currently being studied in children (ClinicalTrials.gov Identifier NCT03587272).¹²⁶ Regardless of the approach taken, any patient with SCD and a history of stroke or other cerebrovascular disease should be evaluated for HSCT at a center with experience treating SCD.

Investigational Therapies

Voxelotor and Crizanlizumab

Ongoing clinical trials are evaluating voxelotor and crizanlizumab, disease-modifying drugs recently approved in the United States for SCD management, for their effects on cerebrovascular complications (Table 4). Crizanlizumab (SelG1) downregulates endothelial expression of P-selectin, thereby reducing inflammation-mediated cell adhesion.^{15,127,128} In the Study to Assess Safety and Impact of SelG1 with or Without Hydroxyurea Therapy in Sickle Cell Disease Patients

Table 4 Active Trials of Disease-Modifying Therapy for Cerebrovascular Disease in the United States

ID	Drug	Design	Outcome	Ages (Years)	Sponsor
NCT04218084	Voxelotor	RCT	TCD	2 to 14	Global Blood Therapeutics
NCT05228834		RCT	Neurocognition	8 to 17	Global Blood Therapeutics
NCT02850406		Single-arm	Pharmacokinetics, hemoglobin, TCD	0.5 to 17	Global Blood Therapeutics
NCT05018728		Single-arm	Hemodynamics	4 to 17	Robert Clark Brown
NCT05334576	Crizanlizumab	Single-arm	SCI	16 and older	Andria Ford

Abbreviations: ID, ClinicalTrials.gov Identification Number; RCT, randomized controlled trial; TCD, transcranial Doppler; SCI, silent cerebral infarction.

Table 5 Active Trials of Gene Therapy for Sickle Cell Disease in the United States

ID	Type	Ages (Years)	Cerebral Vasculopathy	Sponsor
NCT04293185	Gene insertion	2 to 50	Excluded	Bluebird bio., Inc.
NCT05353647		13 to 40	Excluded	David Williams, NHLBI, CIRM, BMTCTN
NCT02247843		≥18	Included	Donald B. Kohn, CIRM
NCT04819841	Gene editing	12 to 40	Included	Graphite Bio, Inc.
NCT04853576		18 to 50	Excluded	Editas Medicine, Inc.
NCT05456880		18 to 35	Excluded	Beam Therapeutics, Inc.
NCT05329649		2 to 11	Included	Vertex Pharmaceuticals, Inc.
NCT05477563		12 to 35	Excluded	Vertex Pharmaceuticals, Inc.
NCT04443907		2 to 40	Excluded	Novartis Pharmaceuticals

Abbreviations: ID, ClinicalTrials.gov Identification Number; NHLBI, National Heart, Lung, and Blood Institute; CIRM, California Institute for Regenerative Medicine; BMTCTN, Bone Marrow Transplantation Clinical Trials Network.

with Pain Crises (SUSTAIN), crizanlizumab decreased vaso-occlusive episodes and acute chest syndrome; there was one stroke in the study group, but cerebrovascular complications were not a primary or secondary endpoint.¹²⁹

Voxelotor stabilizes HbS in the oxygenated state, which inhibits polymerization and erythrocyte sickling.^{127,130} Voxelotor is approved in the United States for patients with SCD at least 12 years of age; the Hemoglobin Oxygen Affinity Modulation to Inhibit HbS Polymerization (HOPE) study did not measure stroke incidence.¹³¹ Stroke was a concern during the development of the drug, due to initial concerns that increasing oxygen affinity would reduce oxygen extraction in sensitive tissues like the brain.¹³² The HOPE trial did not report any strokes in the control or voxelotor groups, nor did interim analysis of HOPE Kids (ClinicalTrials.gov Identifier NCT02850406), an ongoing pediatric voxelotor trial that includes TCD velocity as a primary endpoint.¹³³ TCD velocity is also the primary study question of the HOPE Kids 2 study (ClinicalTrials.gov Identifier NCT04218084).

Gene Therapy

As a monogenic disorder caused by a point mutation, SCD has long been an appealing target for gene therapy.¹³⁴ Current approaches to gene therapy involve collecting a patient’s hematopoietic progenitor cells via marrow harvest or apheresis; genetically modifying the cells ex vivo by inserting a new transgene or, more recently, direct gene editing; and reinfusing the genetically-modified progenitors after high-dose chemotherapy conditioning.¹³⁵ In contrast to allogeneic HSCT, there is no need for a donor and no risk of GVHD. Although gene therapy figures prominently in recent media coverage of SCD,^{136,137} this approach to treatment is investigational, and its risks and benefits are still being established. As of November, 2022 multiple publicly- and privately-funded studies of gene therapy for SCD were actively recruiting in the United States; most of these studies exclude people with cerebrovascular disease (Table 5).

Ongoing research will be necessary to evaluate the potential risk and benefits of gene therapy for reducing stroke risk. The largest clinical trial of gene therapy for SCD to date was conducted by the biotechnology corporation bluebird bio, using transgene insertion.¹³⁸ Among 35 participants, there was sustained improvement in hemoglobin concentrations, with decreased vaso-occlusive events and no strokes after treatment, including participants with a prior history of stroke. Two participants subsequently developed worsening anemia and cytogenetic abnormalities, and one participant developed leukemia five years after gene therapy.^{138,139} In addition to short- and long-term toxicities of conditioning chemotherapy, gene therapy can potentially lead to “genotoxicity”, or malignant transformation of the genetically-modified progenitors,^{140,141} emphasizing the imperative for long-term follow-up research in this promising field.

Conclusion

Stroke is a devastating contributor to mortality, disability, and loss of neurocognitive function for people with SCD. With access to effective TCD screening and chronic transfusion therapy, which can be substituted for hydroxyurea in selected cases, people with SCD can avoid these complications. Stroke prophylaxis contributes to the overall burden of illness, requiring lifelong management of secondary complications like iron overload, as well as monitoring for neurocognitive complications, vascular malformations, and SCI. People with SCD and cerebrovascular disease can have complex medical and social needs, which require lifelong, comprehensive, patient-centered, multidisciplinary care. Screening and prophylaxis are not available to all people with SCD, due in part to structural disparities in healthcare that must be addressed through policy and advocacy.

There is an ongoing need for research into cerebrovascular complications of SCD, both to address the burdens and limitations of standard treatment and to develop new treatments in a safe but expeditious manner. The past several years have seen a sharp increase in new therapies for SCD, and ongoing clinical trials will evaluate the efficacy of medications like voxelotor and crizanlizumab for stroke prevention. Concurrently, clinical trials in HSCT have the potential to further increase the availability of safe, effective, curative therapy for people with SCD. Gene therapy is a promising area of research and development, which is drawing attention from the media and public; both the long-term benefits of gene therapy for people with cerebrovascular complications of SCD and the long-term risks of genetically modifying marrow progenitors require ongoing study.

As we look toward the future of SCD management, we must not lose sight of the immense suffering caused by cerebrovascular disease for people with SCD and their families; the racial, economic, and geographic barriers to equitable management of these complications; and, above all, the fact that collaboration between policymakers, non-governmental organizations, industry, researchers, medical providers, and people with SCD can overcome this devastating burden of illness.

Disclosure

Dr Maria Boucher reports personal fees from Forma, personal fees from GBT, outside the submitted work. The authors report no conflict of interest in this work.

References

1. Allison AC. Notes on sickle-cell polymorphism. *Ann Hum Genet.* 1954;19(1):39–51. doi:10.1111/j.1469-1809.1954.tb01261.x
2. Piel FB, Patil AP, Howes RE, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nat Commun.* 2010;1:104. doi:10.1038/ncomms1104
3. Esoh K, Wonkam A. Evolutionary history of sickle-cell mutation: implications for global genetic medicine. *Hum Mol Genet.* 2021;30(R1):R119–R128. doi:10.1093/hmg/ddab004
4. Wailoo K. *Drawing Blood: Technology and Disease Identity in Twentieth-Century America.* Johns Hopkins University Press; 1999.
5. Steinberg MH. Pathophysiology of sickle cell disease. *Baillieres Clin Haematol.* 1998;11(1):163–184. doi:10.1016/s0950-3536(98)80074-7
6. Carden MA, Fasano RM, Meier ER. Not all red cells sickle the same: contributions of the reticulocyte to disease pathology in sickle cell anemia. *Blood Rev.* 2020;40:100637. doi:10.1016/j.blre.2019.100637
7. Hebbel RP. Ischemia-reperfusion injury in sickle cell anemia: relationship to acute chest syndrome, endothelial dysfunction, arterial vasculopathy, and inflammatory pain. *Hematol Oncol Clin North Am.* 2014;28(2):181–198. doi:10.1016/j.hoc.2013.11.005
8. Kuypers FA. Hemoglobin s polymerization and red cell membrane changes. *Hematol Oncol Clin North Am.* 2014;28(2):155–179. doi:10.1016/j.hoc.2013.12.002
9. Nouraie M, Lee JS, Zhang Y, et al. The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe. *Haematologica.* 2013;98(3):464–472. doi:10.3324/haematol.2012.068965
10. Liguoro I, Arigliani M, Tan HL, Gupta A. The burden of sleep disordered breathing in children with sickle cell disease. *Pediatr Pulmonol.* 2021;56(12):3607–3633. doi:10.1002/ppul.25632
11. Tantawy A, El-Sherif N, Makkeyah S, et al. Sleep disordered breathing and its relation to stroke and pulmonary hypertension in children with sickle cell disease: a single-center cross-sectional study. *Ann Hematol.* 2023;102(2):271–281. doi:10.1007/s00277-023-05099-4
12. Vingert B, Tamagne M, Habibi A, et al. Phenotypic differences of CD4(+) T cells in response to red blood cell immunization in transfused sickle cell disease patients. *Eur J Immunol.* 2015;45(6):1868–1879. doi:10.1002/eji.201445187
13. van Beers EJ, Yang Y, Raghavachari N, et al. Iron, inflammation, and early death in adults with sickle cell disease. *Circ Res.* 2015;116(2):298–306. doi:10.1161/CIRCRESAHA.116.304577
14. Hoppe CC. Inflammatory mediators of endothelial injury in sickle cell disease. *Hematol Oncol Clin North Am.* 2014;28(2):265–286. doi:10.1016/j.hoc.2013.11.006

15. Kutlar A, Embury SH. Cellular adhesion and the endothelium: p-selectin. *Hematol Oncol Clin North Am*. 2014;28(2):323–339. doi:10.1016/j.hoc.2013.11.007
16. Piccin A, Murphy C, Eakins E, et al. Circulating microparticles, protein C, free protein S and endothelial vascular markers in children with sickle cell anaemia. *J Extracell Vesicles*. 2015;4:28414. doi:10.3402/jev.v4.28414
17. Piccin A, Murphy C, Eakins E, et al. Insight into the complex pathophysiology of sickle cell anaemia and possible treatment. *Eur J Haematol*. 2019;102(4):319–330. doi:10.1111/ejh.13212
18. Lim MY, Ataga KI, Key NS. Hemostatic abnormalities in sickle cell disease. *Curr Opin Hematol*. 2013;20(5):472–477. doi:10.1097/MOH.0b013e328363442f
19. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: new approaches to an old problem. *Hematology*. 2007;2007(1):91–96. doi:10.1182/asheducation-2007.1.91
20. Runge A, Brazel D, Pakbaz Z. Stroke in sickle cell disease and the promise of recent disease modifying agents. *J Neurol Sci*. 2022;442:120412. doi:10.1016/j.jns.2022.120412
21. Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood*. 1998;91(1):288–294.
22. Maduakor C, Alakbarzade V, Sammariaie Y, et al. The epidemiology of neurological complications in adults with sickle cell disease: a retrospective cohort study. *Front Neurol*. 2021;12:744118. doi:10.3389/fneur.2021.744118
23. DeBaun MR, Jordan LC, King AA, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv*. 2020;4(8):1554–1588. doi:10.1182/bloodadvances.2019001142
24. Adams R, McKie V, Nichols F, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. *N Engl J Med*. 1992;326(9):605–610. doi:10.1056/NEJM199202273260905
25. Kwiatkowski JL, Voeks JH, Kanter J, et al. Ischemic stroke in children and young adults with sickle cell disease in the post-STOP era. *Am J Hematol*. 2019;94(12):1335–1343. doi:10.1002/ajh.25635
26. Stotesbury H, Kawadler JM, Hales PW, Saunders DE, Clark CA, Kirkham FJ. Vascular instability and neurological morbidity in sickle cell disease: an integrative framework. *Front Neurol*. 2019;10:871. doi:10.3389/fneur.2019.00871
27. Schatz J, White DA, Moinuddin A, Armstrong M, DeBaun MR. Lesion burden and cognitive morbidity in children with sickle cell disease. *J Child Neurol*. 2002;17(12):890–894. doi:10.1177/08830738020170122401
28. Vichinsky EP, Neumayr LD, Gold JI, et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA*. 2010;303(18):1823–1831. doi:10.1001/jama.2010.562
29. Prussien KV, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol*. 2019;44(8):948–958. doi:10.1093/jpepsy/jsz031
30. Portela GT, Butters MA, Brooks MM, Candra L, Rosano C, Novelli EM. Comprehensive assessment of cognitive function in adults with moderate and severe sickle cell disease. *Am J Hematol*. 2022;97(9):E344–E346. doi:10.1002/ajh.26643
31. Adams RJ, Nichols FT, Figueroa R, McKie V, Lott T. Transcranial Doppler correlation with cerebral angiography in sickle cell disease. *Stroke*. 1992;23(8):1073–1077. doi:10.1161/01.str.23.8.1073
32. Siegel MJ, Luker GD, Glauser TA, DeBaun MR. Cerebral infarction in sickle cell disease: transcranial Doppler US versus neurologic examination. *Radiology*. 1995;197(1):191–194. doi:10.1148/radiology.197.1.7568822
33. Silva GS, Vicari P, Figueiredo MS, Carrete H, Idagawa MH, Massaro AR. Brain magnetic resonance imaging abnormalities in adult patients with sickle cell disease: correlation with transcranial Doppler findings. *Stroke*. 2009;40(7):2408–2412. doi:10.1161/STROKEAHA.108.537415
34. Adams RJ, McKie VC, Brambilla D, et al. Stroke prevention trial in sickle cell anemia. *Control Clin Trials*. 1998;19(1):110–129.
35. Hankins JS, Fortner GL, McCarville MB, et al. The natural history of conditional transcranial Doppler flow velocities in children with sickle cell anaemia. *Br J Haematol*. 2008;142(1):94–99. doi:10.1111/j.1365-2141.2008.07167.x
36. Jones AM, Seibert JJ, Nichols FT, et al. Comparison of transcranial color Doppler imaging (TCDI) and transcranial Doppler (TCD) in children with sickle-cell anemia. *Pediatr Radiol*. 2001;31(7):461–469. doi:10.1007/s002470100427
37. McCarville MB, Li C, Xiong X, Wang W. Comparison of transcranial Doppler sonography with and without imaging in the evaluation of children with sickle cell anemia. *Am J Roentgenol*. 2004;183(4):1117–1122. doi:10.2214/ajr.183.4.1831117
38. Lee L, Smith-Whitley K, Banks S, Puckrein G. Reducing health care disparities in sickle cell disease: a review. *Public Health Rep*. 2019;134(6):599–607. doi:10.1177/0033354919881438
39. Wailoo K. Dying in the city of the blues: sickle cell anemia and the politics of race and health. Studies in social medicine. University of North Carolina Press; 2001. Available from: <http://www.loc.gov/catdir/samples/unc041/00062865.html>. Accessed April 2, 2015.
40. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med*. 1998;339(1):5–11. doi:10.1056/NEJM199807023390102
41. Lee MT, Piomelli S, Granger S, et al. Stroke Prevention Trial in Sickle Cell Anemia (STOP): extended follow-up and final results. *Blood*. 2006;108(3):847–852. doi:10.1182/blood-2005-10-009506
42. Abboud MR, Yim E, Musallam KM, Adams RJ. Discontinuing prophylactic transfusions increases the risk of silent brain infarction in children with sickle cell disease: data from STOP II. *Blood*. 2011;118(4):894–898. doi:10.1182/blood-2010-12-326298
43. Wayne AS, Kevy SV, Nathan DG. Transfusion management of sickle cell disease. *Blood*. 1993;81(5):1109–1123.
44. Wahl S, Quirolo KC. Current issues in blood transfusion for sickle cell disease. *Curr Opin Pediatr*. 2009;21(1):15–21. doi:10.1097/MOP.0b013e328328321882e
45. Marouf R. Blood transfusion in sickle cell disease. *Hemoglobin*. 2011;35(5–6):495–502. doi:10.3109/03630269.2011.596984
46. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. *Blood Adv*. 2020;4(2):327–355. doi:10.1182/bloodadvances.2019001143
47. Howard J. Sickle cell disease: when and how to transfuse. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):625–631.
48. Talano JAM, Hillery CA, Gottschall JL, Baylerian DM, Scott JP. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. *Pediatrics*. 2003;111(6 Pt 1):e661–665.
49. Win N. Hyperhemolysis syndrome in sickle cell disease. *Expert Rev Hematol*. 2009;2(2):111–115. doi:10.1586/ehm.09.2

50. Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood*. 2012;120(3):528–537. doi:10.1182/blood-2011-11-327361
51. Linder GE, Chou ST. Red cell transfusion and alloimmunization in sickle cell disease. *Haematologica*. 2021;106(7):1805–1815. doi:10.3324/haematol.2020.270546
52. LaSalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). *Transfusion*. 2011;51(8):1732–1739. doi:10.1111/j.1537-2995.2010.03045.x
53. Casas J, Friedman DF, Jackson T, Vege S, Westhoff CM, Chou ST. Changing practice: red blood cell typing by molecular methods for patients with sickle cell disease. *Transfusion*. 2015. doi:10.1111/trf.12987
54. Coates TD, Wood JC. How we manage iron overload in sickle cell patients. *Br J Haematol*. 2017;177(5):703–716. doi:10.1111/bjh.14575
55. Wilson SR, Sears M, Williams E, et al. Gaps in the diagnosis and management of iron overload in sickle cell disease: a “real-world” report from the GRNDaD registry. *Br J Haematol*. 2021;195(5):e157–e160. doi:10.1111/bjh.17762
56. Adamkiewicz TV, Abboud MR, Paley C, et al. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are nonlinear and are associated with iron load and liver injury. *Blood*. 2009;114(21):4632–4638. doi:10.1182/blood-2009-02-203323
57. Wood JC. Estimating tissue iron burden: current status and future prospects. *Br J Haematol*. 2015;170(1):15–28. doi:10.1111/bjh.13374
58. Vichinsky E, Onyekwere O, Porter J, et al. A randomised comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. *Br J Haematol*. 2007;136(3):501–508. doi:10.1111/j.1365-2141.2006.06455.x
59. Vichinsky E, Pakbaz Z, Onyekwere O, et al. Patient-reported outcomes of deferasirox (Exjade, ICL670) versus deferoxamine in sickle cell disease patients with transfusional hemosiderosis. Substudy of a randomized open-label Phase II trial. *Acta Haematol*. 2008;119(3):133–141. doi:10.1159/000125550
60. Treadwell MJ, Law AW, Sung J, et al. Barriers to adherence of deferoxamine usage in sickle cell disease. *Pediatr Blood Cancer*. 2005;44(5):500–507. doi:10.1002/pbc.20290
61. Novartis. Exjade (Deferasirox). United States Food and Drug Administration; 2013. Available from: https://www.accessdata.fda.gov/drug_satfda_docs/label/2013/021882s019lbl.pdf. Accessed November 21, 2022.
62. Velasquez J, Wray AA. Deferoxamine. In: *StatPearls*. StatPearls Publishing; 2022. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK557654/>. Accessed November 21, 2022.
63. Calvaruso G, Vitranò A, Di Maggio R, et al. Deferiprone versus deferoxamine in sickle cell disease: results from a 5-year long-term Italian multi-center randomized clinical trial. *Blood Cells Mol Dis*. 2014;53(4):265–271. doi:10.1016/j.bcmd.2014.04.004
64. Elalfy MS, Hamdy M, El Beshlawy A, et al. Deferiprone for transfusional iron overload in sickle cell disease and other anemias: open-label study of up to 3 years. *Blood Adv*. 2022;bloodadvances.2021006778. doi:10.1182/bloodadvances.2021006778
65. Ware RE, Davis BR, Schultz WH, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial Doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWITCH): a multicentre, open-label, phase 3, non-inferiority trial. *Lancet*. 2016;387(10019):661–670. doi:10.1016/S0140-6736(15)01041-7
66. Treadwell MJ, Du L, Bhasin N, et al. Barriers to hydroxyurea use from the perspectives of providers, individuals with sickle cell disease, and families: report from a U.S. regional collaborative. *Front Genet*. 2022;13:921432. doi:10.3389/fgene.2022.921432
67. Vick L, Potts M, Jaskowiak M, Gibson RW. Hydroxyurea adherence strategies for persons with sickle cell disease: a systematic review. *J Health Care Poor Underserved*. 2021;32(1):99–118. doi:10.1353/hpu.2021.0011
68. Hodges JR, Phillips SM, Norell S, et al. Intentional and unintentional nonadherence to hydroxyurea among people with sickle cell disease: a qualitative study. *Blood Adv*. 2020;4(18):4463–4473. doi:10.1182/bloodadvances.2020001701
69. Steinberg MH, McCarthy WF, Castro O, et al. The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: a 17.5 year follow-up. *Am J Hematol*. 2010;85(6):403–408. doi:10.1002/ajh.21699
70. Rodriguez A, Duez P, Dedeken L, Cotton F, Ferster A. Hydroxyurea (hydroxycarbamide) genotoxicity in pediatric patients with sickle cell disease. *Pediatr Blood Cancer*. 2018;65(7):e27022. doi:10.1002/pbc.27022
71. Rankine-Mullings AE, Nevitt SJ. Hydroxyurea (hydroxycarbamide) for sickle cell disease. *Cochrane Database Syst Rev*. 2022;(9). doi:10.1002/14651858.CD002202.pub3
72. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet*. 2011;377(9778):1663–1672. doi:10.1016/S0140-6736(11)60355-3
73. Lebensburger JD, Miller ST, Howard TH, et al. Influence of severity of anemia on clinical findings in infants with sickle cell anemia: analyses from the BABY HUG study. *Pediatr Blood Cancer*. 2012;59(4):675–678. doi:10.1002/pbc.24037
74. Smart LR, Ambrose EE, Balyorugulu G, et al. Stroke Prevention with Hydroxyurea Enabled through Research and Education (SPHERE): a Phase 2 primary stroke prevention trial in Sub-Saharan Africa. *AHA*. 2022;145(6):66. doi:10.1159/000526322
75. Miller ST, Macklin EA, Pegelow CH, et al. Silent infarction as a risk factor for overt stroke in children with sickle cell anemia: a report from the Cooperative Study of Sickle Cell Disease. *The Journal of Pediatrics*. 2001;139(3):385–390. doi:10.1067/mpd.2001.117580
76. DeBaun MR, Armstrong FD, McKinstry RC, Ware RE, Vichinsky E, Kirkham FJ. Silent cerebral infarcts: a review on a prevalent and progressive cause of neurologic injury in sickle cell anemia. *Blood*. 2012;119(20):4587–4596. doi:10.1182/blood-2011-02-272682
77. DeBaun MR, Gordon M, McKinstry RC, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *N Engl J Med*. 2014;371(8):699–710. doi:10.1056/NEJMoa1401731
78. Guillems KP, Fields ME, Hulbert ML. Higher-than-expected prevalence of silent cerebral infarcts in children with hemoglobin SC disease. *Blood*. 2015;125(2):416–417. doi:10.1182/blood-2014-10-605964
79. Sathi BK, Yoshida Y, Weaver MR, et al. Unusually high prevalence of stroke and cerebral vasculopathy in hemoglobin SC Disease: a Retrospective Single Institution Study. *Acta Haematol*. 2022;145(2):160–169. doi:10.1159/000519360
80. Hasson C, Veling L, Rico J, Mhaskar R. The role of hydroxyurea to prevent silent stroke in sickle cell disease. *Medicine*. 2019;98(51):e18225. doi:10.1097/MD.00000000000018225
81. Fields ME, Guillems KP, Ragan D, et al. Hydroxyurea reduces cerebral metabolic stress in patients with sickle cell anemia. *Blood*. 2019;133(22):2436–2444. doi:10.1182/blood-2018-09-876318
82. Estcourt LJ, Kimber C, Hopewell S, Trivella M, Doree C, Abboud MR. Interventions for preventing silent cerebral infarcts in people with sickle cell disease. *Cochrane Database Syst Rev*. 2020;4:CD012389. doi:10.1002/14651858.CD012389.pub3

83. Powars D, Wilson B, Imbus C, Pegelow C, Allen J. The natural history of stroke in sickle cell disease. *Am J Med.* 1978;65(3):461–471.
84. Webb J, Kwiatkowski JL. Stroke in patients with sickle cell disease. *Expert Rev Hematol.* 2013;6(3):301–316. doi:10.1586/ehm.13.25
85. Rafay MF, Shapiro KA, Surmava AM, et al. Spectrum of cerebral arteriopathies in children with arterial ischemic stroke. *Neurology.* 2020;94(23):e2479–e2490. doi:10.1212/WNL.00000000000009557
86. Aa K, Na G. How I treat and manage strokes in sickle cell disease. *Blood.* 2015;125(22). doi:10.1182/blood-2014-09-551564
87. Hulbert ML, Scothorn DJ, Panepinto JA, et al. Exchange blood transfusion compared with simple transfusion for first overt stroke is associated with a lower risk of subsequent stroke: a retrospective cohort study of 137 children with sickle cell anemia. *J Pediatr.* 2006;149(5):710–712. doi:10.1016/j.jpeds.2006.06.037
88. Hurler-Jensen AM, Prohovnik I, Pavlakis SG, Piomelli S. Effects of total hemoglobin and hemoglobin S concentration on cerebral blood flow during transfusion therapy to prevent stroke in sickle cell disease. *Stroke.* 1994;25(8):1688–1692. doi:10.1161/01.str.25.8.1688
89. Strouse JJ, Lanzkron S, Urrutia V. The epidemiology, evaluation and treatment of stroke in adults with sickle cell disease. *Expert Rev Hematol.* 2011;4(6):597–606. doi:10.1586/ehm.11.61
90. Frye RE. Reversible posterior leukoencephalopathy syndrome in sickle-cell anemia. *Pediatr Neurol.* 2009;40(4):298–301. doi:10.1016/j.pediatrneurol.2008.10.024
91. Hinchey J, Chaves C, Appignani B, et al. A reversible posterior leukoencephalopathy syndrome. *N Engl J Med.* 1996;334(8):494–500. doi:10.1056/NEJM19960223340803
92. Raj S, Killinger J, Overby P. Blood transfusion in sickle cell disease leading to posterior reversible encephalopathy syndrome (PRES). *J Child Neurol.* 2013;28(10):1284–1286. doi:10.1177/0883073812453497
93. Vargas A, Testai FD. Posterior reversible encephalopathy syndrome in adult sickle-cell patients: case series and literature review. *J Clin Neurosci.* 2019;70:249–250. doi:10.1016/j.jocn.2019.08.070
94. Sidani CA, Ballourah W, El Dassouki M, et al. Venous sinus thrombosis leading to stroke in a patient with sickle cell disease on hydroxyurea and high hemoglobin levels: treatment with thrombolysis. *Am J Hematol.* 2008;83(10):818–820. doi:10.1002/ajh.21261
95. Rahimi Z, Parsian A. Sickle cell disease and venous thromboembolism. *Mediterr J Hematol Infect Dis.* 2011;3(1):e2011024. doi:10.4084/MJHID.2011.024
96. Lusher JM, Haghighat H, Khalifa AS. A prophylactic transfusion program for children with sickle cell anemia complicated by CNS infarction. *Am J Hematol.* 1976;1(2):265–273. doi:10.1002/ajh.2830010210
97. Russell M, Goldberg H, Hodson A, et al. Effect of transfusion therapy on arteriographic abnormalities and on recurrence of stroke in sickle cell disease. *Blood.* 1984;63(1):162–169. doi:10.1182/blood.V63.1.162.162
98. Pegelow CH, Adams RJ, McKie V, et al. Risk of recurrent stroke in patients with sickle cell disease treated with erythrocyte transfusions. *J Pediatr.* 1995;126(6):896–899. doi:10.1016/S0022-3476(95)70204-0
99. Estcourt LJ, Kohli R, Hopewell S, Trivella M, Wang WC. Blood transfusion for preventing primary and secondary stroke in people with sickle cell disease. *Cochrane Database Syst Rev.* 2020;7:CD003146. doi:10.1002/14651858.CD003146.pub4
100. Ware RE, Helms RW. Stroke With Transfusions Changing to Hydroxyurea (SWiTCH). *Blood.* 2012;119(17):3925–3932. doi:10.1182/blood-2011-11-392340
101. Kayle M, Docherty SL, Sloane R, et al. Transition to adult care in sickle cell disease: a longitudinal study of clinical characteristics and disease severity. *Pediatr Blood Cancer.* 2019;66(1):e27463. doi:10.1002/pbc.27463
102. Lanzkron S, Carroll CP, Haywood C. Mortality rates and age at death from sickle cell disease: u.S., 1979–2005. *Public Health Rep.* 2013;128(2):110–116. doi:10.1177/003335491312800206
103. Anie KA, Telfair J; Sickle Cell Disease Transition Study Working Group. Multi-site study of transition in adolescents with sickle cell disease in the United Kingdom and the United States. *Int J Adolesc Med Health.* 2005;17(2):169–178. doi:10.1515/ijamh.2005.17.2.169
104. Treadwell M, Telfair J, Gibson RW, Johnson S, Osunkwo I. Transition from pediatric to adult care in sickle cell disease: establishing evidence-based practice and directions for research. *Am J Hematol.* 2011;86(1):116–120. doi:10.1002/ajh.21880
105. Treadwell M, Johnson S, Sisler I, et al. Development of a sickle cell disease readiness for transition assessment. *Int J Adolesc Med Health.* 2016;28(2):193–201. doi:10.1515/ijamh-2015-0010
106. Currie S, Raghavan A, Batty R, Connolly DJA, Griffiths PD. Childhood moyamoya disease and moyamoya syndrome: a pictorial review. *Pediatr Neurol.* 2011;44(6):401–413. doi:10.1016/j.pediatrneurol.2011.02.007
107. Dobson SR, Holden KR, Nietert PJ, et al. Moyamoya syndrome in childhood sickle cell disease: a predictive factor for recurrent cerebrovascular events. *Blood.* 2002;99(9):3144–3150.
108. Griessenauer CJ, Lebensburger JD, Chua MH, et al. Encephaloduroarteriosynangiosis and encephalomyoarteriosynangiosis for treatment of moyamoya syndrome in pediatric patients with sickle cell disease. *J Neurosurg Pediatr.* 2015;1–10. doi:10.3171/2014.12.PEDS14522
109. Winstead M, Sun PP, Martin K, et al. Encephaloduroarteriosynangiosis (EDAS) in young patients with cerebrovascular complications of sickle cell disease: single-institution experience. *Pediatr Hematol Oncol.* 2017;34(2):100–106. doi:10.1080/08880018.2017.1313917
110. Alamri A, Hever P, Cheserem J, Gradil C, Bassi S, Tolias CM. Encephaloduroarteriosynangiosis (EDAS) in the management of Moyamoya syndrome in children with sickle cell disease. *Br J Neurosurg.* 2019;33(2):161–164. doi:10.1080/02688697.2017.1339227
111. Gluckman E, Cappelli B, Bernaudin F, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood.* 2016. doi:10.1182/blood-2016-10-745711
112. Khemani K, Katoch D, Krishnamurti L. Curative therapies for sickle cell disease. *Ochsner J.* 2019;19(2):131–137. doi:10.31486/toj.18.0044
113. Verlhac S, Gabor F, Paillard P, et al. Improved stenosis outcome in stroke-free sickle cell anemia children after transplantation compared to chronic transfusion. *Br J Haematol.* 2021;193(1). doi:10.1111/bjh.17178
114. Thurn S, Kleinschmidt K, Kovacic I, et al. Transcranial Doppler sonography and the effect of haematopoietic stem cell transplantation in sickle cell disease. *Neurol Res Pract.* 2022;4(1):12. doi:10.1186/s42466-022-00175-y
115. Wailoo K, Pemberton S. *The Troubled Dream of Genetic Medicine: Ethnicity and Innovation in Tay-Sachs, Cystic Fibrosis, and Sickle Cell Disease.* Johns Hopkins University Press; 2006. Available from: <http://ebookcentral.proquest.com/lib/unc/detail.action?docID=3318266>. Accessed March 18, 2022.
116. DeBaun MR, Clayton EW. Primum non nocere: the case against transplant for children with sickle cell anemia without progressive end-organ disease. *Blood Adv.* 2017;1(26):2568–2571. doi:10.1182/bloodadvances.2017007690

117. Fitzhugh CD, Walters MC. The case for HLA-identical sibling hematopoietic stem cell transplantation in children with symptomatic sickle cell anemia. *Blood Adv.* 2017;1(26):2563–2567. doi:10.1182/bloodadvances.2017007708
118. Stenger E, Xiang Y, Wetzel M, et al. Long-term organ function after HCT for SCD: a report from the sickle cell transplant advocacy and research alliance. *Transplant Cell Ther.* 2022:S2666-6367(22)01708–0. doi:10.1016/j.jct.2022.10.012
119. Shenoy S, Eapen M, Panepinto JA, et al. A trial of unrelated donor marrow transplantation for children with severe sickle cell disease. *Blood.* 2016;128(21):2561–2567. doi:10.1182/blood-2016-05-715870
120. Ngwube A, Shah N, Godder K, Jacobsohn D, Hulbert ML, Shenoy S. Abatacept is effective as GVHD prophylaxis in unrelated donor stem cell transplantation for children with severe sickle cell disease. *Blood Adv.* 2020;4(16):3894–3899. doi:10.1182/bloodadvances.2020002236
121. Kasow KA, Krajewski J, Haines H, et al. Curing children with sickle hemoglobinopathies of varying severity early in life with HLA-matched sibling donor (MSD) hematopoietic cell transplantation (HCT) following a reduced intensity conditioning (RIC) regimen: a Sickle Transplant, Advocacy, and Research (STAR) trial. *Transplant Cell Ther.* 2021;29(2S):S293.
122. Krishnamurti L, Neuberger DS, Sullivan KM, et al. Bone marrow transplantation for adolescents and young adults with sickle cell disease: results of a prospective multicenter pilot study. *Am J Hematol.* 2019;94(4):446–454. doi:10.1002/ajh.25401
123. Stimpson SJ, DeBaun M. Comparing outcomes for young adults with severe SCD treated with HLA-identical sibling and matched unrelated HCT versus standard supportive care. *Hematologist.* 2016;13:3. doi:10.1182/hem.V13.3.5469
124. Hsieh MM, Fitzhugh CD, Weitzel RP, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. *JAMA.* 2014;312(1):48–56. doi:10.1001/jama.2014.7192
125. Saraf SL, Oh AL, Patel PR, et al. Nonmyeloablative stem cell transplantation with alemtuzumab/low-dose irradiation to cure and improve the quality of life of adults with sickle cell disease. *Biol Blood Marrow Transplant.* 2016;22(3):441–448. doi:10.1016/j.bbmt.2015.08.036
126. Nickel RS, Chiang K, Hardy SJ, et al. Nonmyeloablative HLA-identical sibling donor transplantation for children and young adults with sickle cell disease: interim results of the SUN multicenter phase II trial. *Blood.* 2021;138:1799. doi:10.1182/blood-2021-144454
127. Carden MA, Little J. Emerging disease-modifying therapies for sickle cell disease. *Haematologica.* 2019;104(9):1710–1719. doi:10.3324/haematol.2018.207357
128. Migotsky M, Beestrup M, Badawy SM. Recent advances in sickle-cell disease therapies: a review of voxelotor, crizanlizumab, and L-glutamine. *Pharmacy.* 2022;10(5):123. doi:10.3390/pharmacy10050123
129. Ataga KI, Kutlar A, Kanter J, et al. SUSTAIN: a multicenter, randomized, placebo-controlled, double-blind, 12-month study to assess safety and efficacy of SelG1 with or without hydroxyurea therapy in sickle cell disease patients with sickle cell-related pain crises. *Blood.* 2016;128(22):1. doi:10.1182/blood.V128.22.1.1
130. Safo MK, Kato GJ. Therapeutic strategies to alter the oxygen affinity of sickle hemoglobin. *Hematol Oncol Clin North Am.* 2014;28(2):217–231. doi:10.1016/j.hoc.2013.11.001
131. Ki JH, Re A. Voxelotor in adolescents and adults with sickle cell disease (HOPE): long-term follow-up results of an international, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Haematol.* 2021;8:5. doi:10.1016/S2352-3026(21)00059-4
132. Hebbel RP, Hedlund BE. Sickle hemoglobin oxygen affinity-shifting strategies have unequal cerebrovascular risks. *Am J Hematol.* 2018;93(3):321–325. doi:10.1002/ajh.24975
133. Estep JH, Kalpathi R, Woods G, et al. Safety and efficacy of voxelotor in pediatric patients with sickle cell disease aged 4 to 11 years. *Pediatr Blood Cancer.* 2022;69(8):e29716. doi:10.1002/pbc.29716
134. Bank A, Markowitz D, Lerner N. Gene transfer. A potential approach to gene therapy for sickle cell disease. *Ann N Y Acad Sci.* 1989;565:37–43. doi:10.1111/j.1749-6632.1989.tb24147.x
135. Abraham AA, Tisdale JF. Gene therapy for sickle cell disease: moving from the bench to the bedside. *Blood.* 2021;138(11):932–941. doi:10.1182/blood.2019003776
136. Kolata G. Pioneering gene therapy freed her of sickle cell. Is a cure at hand? *The New York Times*; 2021. Available from: <https://www.nytimes.com/2021/09/14/health/sickle-cell-cure.html>. Accessed November 22, 2022.
137. Stein R. First sickle cell patient treated with CRISPR gene-editing still thriving. *NPR*; 2021. Available from: <https://www.npr.org/sections/health-shots/2021/12/31/1067400512/first-sickle-cell-patient-treated-with-crispr-gene-editing-still-thriving>. Accessed November 22, 2022.
138. Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of lentiglobin for sickle cell disease. *N Engl J Med.* 2022;386(7):617–628. doi:10.1056/NEJMoa2117175
139. Goyal S, Tisdale J, Schmidt M, et al. Acute myeloid leukemia case after gene therapy for sickle cell disease. *N Engl J Med.* 2022;386(2):138–147. doi:10.1056/NEJMoa2109167
140. Kohn DB, Sadelain M, Glorioso JC. Occurrence of leukaemia following gene therapy of X-linked SCID. *Nat Rev Cancer.* 2003;3(7):477–488. doi:10.1038/nrc1122
141. Jones RJ, DeBaun MR. Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither. *Blood.* 2021;138(11):942–947. doi:10.1182/blood.2021011488



Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines

Hana Qasim, Alaa Rahhal, Ahmed Husain, Abdelkarim Alammora, Khaled Alsa'ed, Ahmed Abdelghafar Masaad Alsayed, Baha Faiyoumi, Leen Maen AbuAfifeh, Mohammad Abu-Tineh, Awni Alshurafa & Mohamed A Yassin

To cite this article: Hana Qasim, Alaa Rahhal, Ahmed Husain, Abdelkarim Alammora, Khaled Alsa'ed, Ahmed Abdelghafar Masaad Alsayed, Baha Faiyoumi, Leen Maen AbuAfifeh, Mohammad Abu-Tineh, Awni Alshurafa & Mohamed A Yassin (2023) Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines, Journal of Blood Medicine, , 295-302, DOI: [10.2147/JBM.S396026](https://doi.org/10.2147/JBM.S396026)

To link to this article: <https://doi.org/10.2147/JBM.S396026>



© 2023 Qasim et al.



Published online: 14 Apr 2023.



Submit your article to this journal



Article views: 481



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines

Hana Qasim^{1,2}, Alaa Rahhal³, Ahmed Husain⁴, Abdelkarim Alammora⁵, Khaled Alsa'ed⁵,
Ahmed Abdelghafar Masaad Alsayed⁵, Baha Faiyoumi⁵, Leen Maen AbuAfifeh⁵, Mohammad Abu-Tineh¹,
Awni Alshurafa¹, Mohamed A Yassin¹

¹Hematology-Oncology Department, National Centre for Cancer Care & Research, Doha, Qatar; ²Department of Internal Medicine, University of Missouri-Kansas City, Kansas City, MO, USA; ³MSc Pharmacy Department, Hamad Medical Corporation, Doha, Qatar; ⁴Infectious Disease Department, Communicable Disease Center, Hamad Medical Corporation, Doha, Qatar; ⁵Internal Medicine Department, Hamad Medical Corporation, Doha, Qatar

Correspondence: Hana Qasim; Khaled Alsa'ed, Email Hanaa.qasem1@gmail.com; KAlsaed@hamad.qa

Background: Immune thrombocytopenia (ITP) is a blood disorder in which antibodies coating platelets cause platelet destruction in the spleen with a resultant low platelet count and an increased tendency for bleeding. Coronavirus disease 2019 (COVID-19) is an illness caused by SARS-CoV-2. Though pneumonia and respiratory failure are major causes of morbidity and mortality, multisystemic complications were identified, including hematological ones. Several ITP relapse cases post-mRNA SARS-CoV-2 vaccines have been reported, and different pathophysiological theories have been proposed.

Purpose: The objective of this study is to identify the causal relationship between mRNA COVID-19 vaccines and ITP relapse, to highlight the longer-term effect of these vaccines on the platelet count more than 6 months after receiving the vaccine, and to identify if there is a statistical difference between Comirnaty and Spikevax vaccines on ITP relapse rate.

Patients and Methods: In this retrospective study, 67 patients with known ITP were followed before and after receiving the mRNA COVID-19 vaccine. The follow-up parameters included platelet counts when available and bleeding symptoms. All patients were adults over 18 years old, with no other identified causes of thrombocytopenia. Forty-seven patients received the Comirnaty vaccine, and 20 patients received the Spikevax vaccine.

Results: Data analysis showed 6% ITP relapse in the first 3 months, and a 10% relapse rate 3–6 months after receiving one of the mRNA COVID-19 vaccines, with no statically significant difference between the two vaccines.

Conclusion: mRNA COVID-19 vaccines increase the risk of ITP relapse and can lead to a prolonged reduction in platelet count in a proportion of ITP patients, with no statistically significant difference between Comirnaty and Spikevax vaccines.

Keywords: ITP, vaccine, relapse, COVID-19

Introduction

Immune thrombocytopenic (ITP) is a syndrome in which platelets become coated with autoantibodies to platelet membrane antigens. In patients with ITP, the mononuclear macrophage system of the spleen is responsible for removing platelets, and with incomplete compensation from the bone marrow, this leads to a decrease in circulating platelets count. This proposition is supported by the prompt improvement of a significant proportion of patients after IVIG therapy and splenectomy.^{1,2} Patients can present with bruising, epistaxis, and mucosal bleeding, with a minority of cases developing major bleeding complications such as gastrointestinal tract bleeding and intracranial hemorrhage. The mRNA COVID vaccine is considered generally safe, with millions of people receiving the vaccine with no serious adverse effects, nevertheless, multiple complications, especially autoimmune phenomena have been reported so far,³ including but not limited to myocarditis, Guillain-Barre syndrome, autoimmune hepatitis, arthritis, and hematological complications like thrombotic thrombocytopenic purpura and immune thrombocytopenia purpura. Most reported ITP cases presented with petechia, bruising, and mucosal bleeding in the first few weeks after receiving the vaccine;^{4,5} and showed improvement

with conventional steroid treatment. Nevertheless, recommendations for ITP patients to receive mRNA COVID vaccine are still under discussion. Few retrospective and prospective studies on ITP patients have shown an increased relapse rate after the COVID-19 vaccine.^{6,7}

Methods

This retrospective study was conducted to check platelet counts in 67 patients known to have immune thrombocytopenia purpura, who are >18-years-old, and not known for any other causes of thrombocytopenia (including congenital thrombocytopenia, malignancy, systemic lupus erythematosus, antiphospholipid syndrome). The platelet count was identified at baseline before receiving the vaccine and within 3 months, 6 months, and after 6 months after receiving the vaccine.

For Comirnaty (Pfizer) vaccine, the second dose was given 21 days after the first dose. For Spikevax (Moderna), the second dose was given 28 days after the first dose.

ITP relapse was defined as a drop in platelet counts to less than $30 \times 10^9/L$ with bleeding symptoms or a need to use rescue therapy.

This study aims to demonstrate the causal relationship between ITP relapse in known ITP patients and the mRNA COVID vaccine and to evaluate the long-term effect of the vaccine on ITP patients >6 months after receiving the second dose of the vaccine.

Data Collection

Data were accessed from the existing medical records and were extracted and analyzed.

The selected clinical and pathological data were obtained from the patient charts and/or database. This data includes demographic data diagnosis, lab results and received treatment.

Data was only accessed by a specific username and password and was stored in the hospital computer.

Statistical Analysis

Data analyses (Figures 1 and 2) were performed using the Statistical Package for Social Sciences program version 28.0 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY). Descriptive statistics were reported in the form of frequencies and percentages for categorical variables and mean $\pm$ standard deviation (SD) for continuous variables. The change in platelets after receiving COVID-19 vaccines was analyzed using the paired-sample *t*-test. Student's *t*-test was used to compare the platelet counts between different vaccines. A *p*-value of <0.05 was used to indicate statistical significance.

Results

Patient Characteristics (Table 1)

We included 67 patients (Table 1), 32 (47.8%) males and 35 (52.2%) females, aged 40 ± 13 years. Forty-seven (70.1%) patients received Comirnaty (Pfizer) COVID vaccine and 20 (29.9%) patients received Spikevax (Moderna) COVID vaccine. All patients received two doses of the vaccine as scheduled with no delay. Twenty-eight (41.8%) patients received prior treatment of Eltrombopag, two (3%) received prior Romiplostim, and 19 (28.4%) patients received prior treatment of Rituximab.

COVID-19 Vaccination Effect on Platelet Count (Figures 1 and 2)

Data analysis showed a relapse rate of 9% (6 patients) in the first 3 months after the mRNA COVID-19 vaccine, 15% (10 patients) in 3–6 months, and a 12% (8 patients) relapse rate more than 6 months after receiving the vaccine (Table 2). The trend in absolute platelets count is illustrated in Table 3. There were no relapse cases discovered between the first and second doses of both vaccines. Data analysis also showed a significant platelets drop of >50% in eight (12%) patients, who did not meet the criteria for ITP relapse within the first 6 months after the vaccine, and four (6%) patients had a long-term effect with a >50% reduction in platelets count more than 6 months after receiving the vaccine (Table 2).

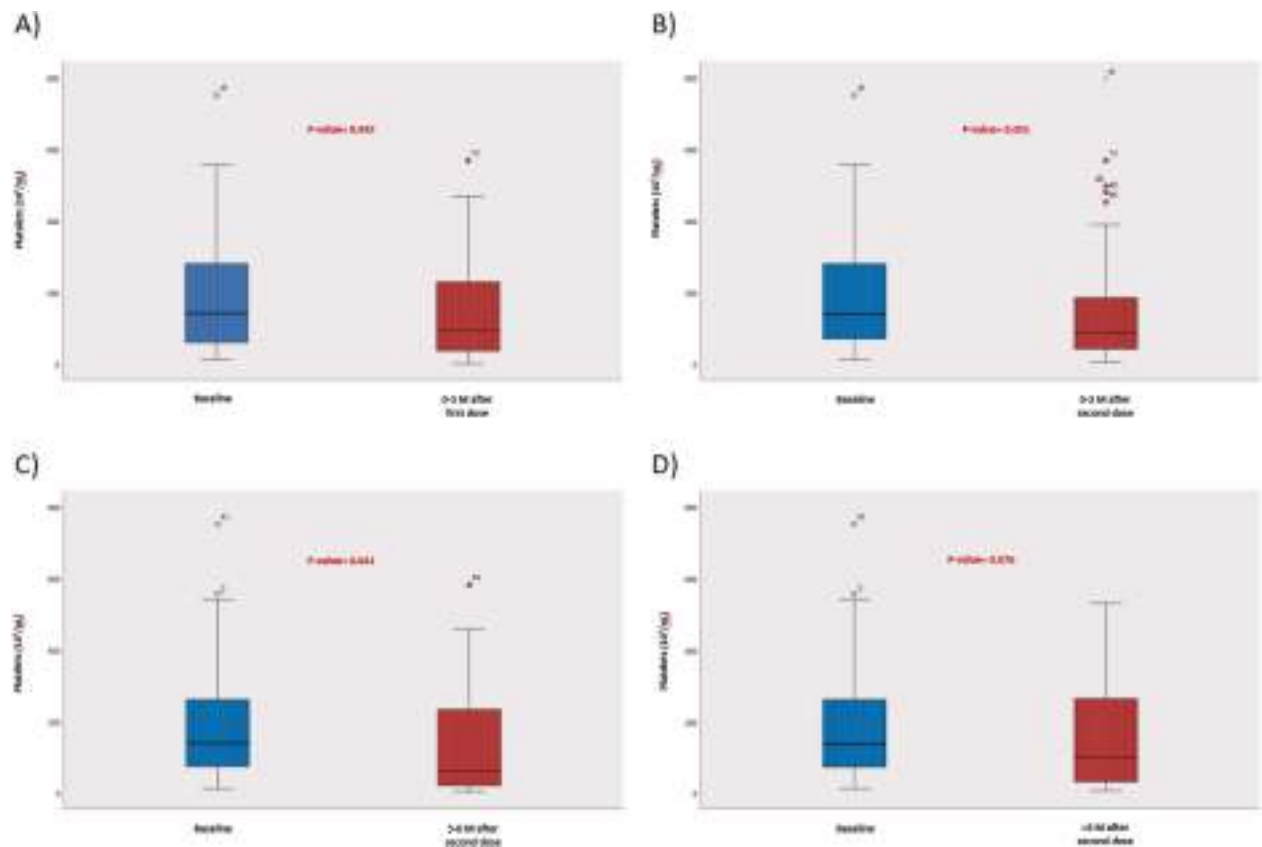


Figure 1 (A-D) The change in platelets after receiving COVID-19 vaccines among patients with ITP.

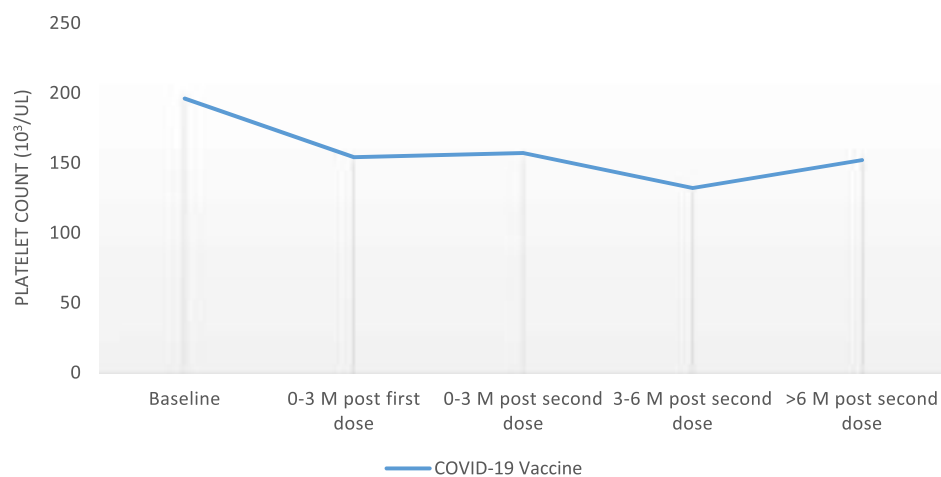


Figure 2 The trend of platelets after receiving COVID-19 vaccines among patients with ITP.

A less dramatic effect on platelet count with a 25–50% drop was observed in 9% of patients within 6 months, and 4.5% more than 6 months after the vaccine. Data analysis did not show a significant difference in ITP relapse cases or platelet number trend between Comirnaty and Spikevax vaccines (Table 4; Figure 3). Of note, two patients had a persistently low platelet count after receiving the vaccine, despite standard ITP treatment. Of the patients who had

Table 1 Baseline Characteristics of Patients with ITP Who Received COVID-19 Vaccines (N=67)

Characteristic	n (%)
Age (years)	40±13
Gender	
Male	32 (47.8)
Female	35 (52.2)
Platelets (10 ³ /μL)	196±154
COVID-19 infection	21 (31.3)
Platelets after COVID-19 infection	213±187
Vaccine	
Comirnaty (Pfizer)	47 (70.1)
Spikevax (Moderna)	20 (29.9)
ITP medications	
Steroids	3 (4.5)
Rituximab	19 (28.4)
Romiplostim	2 (3)
Eltrombopag	28 (41.8)

Table 2 Outcomes of Patients with ITP Who Received COVID-19 Vaccines (N=67)

Outcome	N (%)
ITP relapse within 3 months of vaccination	6 (9)
ITP relapse within 6 months of vaccination	10 (15)
ITP relapse within >6 months of vaccination	8 (12)
25–50% reduction in platelets within 6 months of vaccination	6 (9)
25–50% reduction in platelets >6 months of vaccination	3 (4.5)
>50% reduction in platelets within 6 months of vaccination	8 (12)
>50% reduction in platelets >6 months of vaccination	4 (6)

Table 3 Trend of Platelets After Receiving COVID-19 Vaccines Among Patients with ITP (N=67)

Interval	Platelets Before Vaccine	Platelets After Vaccine	P-value
0–3 months post first dose	196±154	154±152	0.043
0–3 months post second dose	196±154	157±183	0.031
3–6 months post second dose	196±154	132±152	0.041
>6 months post second dose	196±154	152±155	0.076

Table 4 Platelets After Receiving COVID-19 Vaccines Among Patients with ITP (N=67)

Interval	Platelets After Comirnaty Vaccine	Platelets After Spikevax Vaccine	P-value
Before vaccination	194±159	200±145	0.886
0–3 months post first dose	173±155	101±137	0.189
0–3 months post second dose	177±200	100±112	0.133
3–6 months post second dose	150±158	69±112	0.120
>6 months post second dose	160±163	124±131	0.474

ITP relapse after the vaccine, five had a recorded COVID-19 infection, and none of them had evidence of relapse within 1 month of the infection.

We studied the possible predictors of relapse, including gender, age, previous COVID-19 infection, and ITP treatment, using a logistic regression model, which showed no significant effect of any of these factors, as all patients are at the same risk of relapse regardless of these factors (Table 5).

Discussion

In response to the urgent need for effective vaccines to stop the COVID pandemic, the World Health Organization listed both the Comirnaty (Pfizer/BioNTech) vaccine and Spikevax (Moderna) COVID-19 vaccine (mRNA 1273) for emergency use listing on December 31, 2020 and the April 30, 2021, respectively. Both vaccines are mRNA-based vaccines that encode SARS-CoV-2 spike protein and enhance immunogenicity against the virus.^{8,9} There were no major safety concerns related to the vaccines initially,^{10,11} but, over time, there has been increased recognition of post-mRNA SARS-CoV-2 vaccine immune phenomena,^{12,13} including immune thrombocytopenia.^{3,14} With the need to have clear recommendations for ITP patients who are planning to receive the COVID-19 vaccine, we conducted this retrospective study, which has shown an increased relapse rate and a drop in platelets count, which is not limited to the first few weeks after the vaccine, as was thought before. As ITP relapse cases were reported before with older vaccines,^{15–17} there are some proposed potential mechanisms of immune activation triggered by vaccines administration, which can apply to the COVID-19 vaccine, including molecular mimicry,^{3–5} where the immune cross-reactivity triggered by the similarity between certain vaccine components and specific human proteins causes the immune system to attack normal tissue proteins in a susceptible population. Another is by creating a hyper-inflammatory environment, with increased production

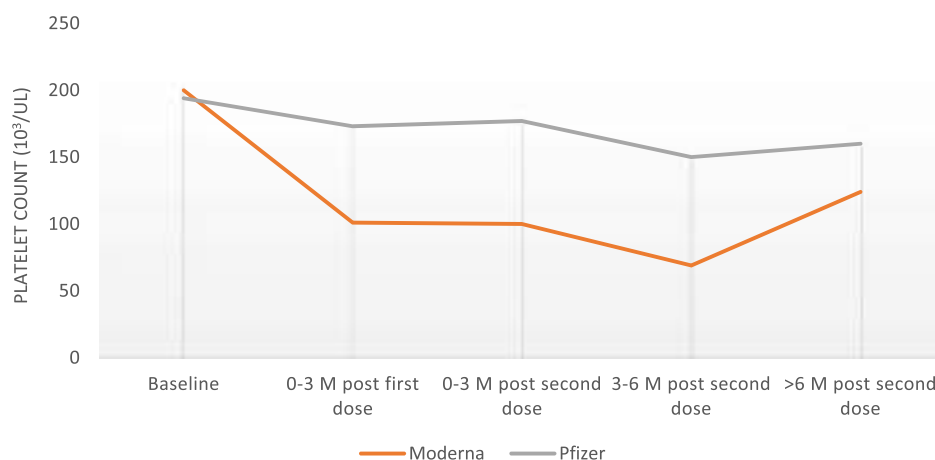
**Figure 3** The trend of platelets after receiving Comirnaty (Pfizer) and Spikevax (Moderna) COVID-19 vaccines among patients with ITP.

Table 5 Predictors of Relapse

Factor	Odds Ratio	95% Confidence Interval	P-value
Male gender	1.3	0.4–4.3	0.651
Age (older than 40 years)	1.6	0.5–5.0	0.435
Comirnaty vaccine	0.6	0.2–2.1	0.442
COVID infection	1.5	0.5–5.1	0.502
Concurrent steroids	1.9	0.1–31.9	0.664
Concurrent TPO-RA	1.0	0.3–3.2	0.955
Rituximab treatment	0.6	0.1–2.7	0.525

of the pro-inflammatory cytokine, resultant oxidative stress, DNA damage in the mononuclear cells, and T-cell dysregulation. The hyperinflammatory environment in bone marrow might lead to megakaryocyte apoptosis and a decrease in platelet count.^{18,19} Though most patients with COVID-19 vaccine-induced relapses respond to first-line treatment with steroids and IVIG, refractory cases were reported.²⁰ The new persistent and likely different autoimmune process, where newly developed antibodies targeting new platelet antigens induced by molecular mimicry, associated with megakaryocyte apoptosis, might explain the treatment resistance that some ITP patients showed after receiving the vaccine.²⁰

A recent multicenter study in the Netherlands showed exacerbation of ITP occurred in 30 of the 218 ITP patients (14%) after COVID-19 vaccination, risk factors for ITP relapse in subgroup analysis included patients with baseline platelet count $< 50 \times 10^9/L$, patients on ITP treatment at the start of COVID-19 vaccination, and patients with younger age. It also demonstrated a 6.3% decrease in platelet count compared with baseline, 4 weeks after the second vaccination.²¹

We found a more significant drop in platelets count from the baseline in patients who did not meet the criteria for ITP relapse (Figure 2), with platelets drop of $>50\%$ in 12% of patients within the first 6 months after the vaccine, and 6% of patients had a long-term effect with $>50\%$ reduction in platelets count more than 6 months after receiving the vaccine. Additionally, our data analysis did not show increased risk related to age, gender, type of treatment received, or previous COVID-19 infection. This does not exclude the possibility of the effect of these factors but might be related to the smaller sample size in our study.

On further follow-up, two patients with baseline platelets of $>100 \times 10^9/L$ had persistently low platelet counts after receiving the vaccine with platelets count $<30 \times 10^9/L$ despite standard ITP treatment, and one of them had splenectomy eventually. We propose that the long-term effect on some patients exceeding the first few weeks after the vaccine and the newly-developed refractory cases might be related to new and persistent autoimmune processes to new antigens in the susceptible patient.

Though up to 85% of ITP patients respond to initial standard treatment with steroids,²² only 15% maintain this response over the next year. In one study where 281 ITP patients were followed for 60 months, 51.6% developed a relapse event, of whom the median time between response to relapse was 1.16 (0.03–96) months.

Second-line therapy such as thrombopoietin receptors agonists, Rituximab, or splenectomy is required in a good proportion of patients to maintain safe platelet levels. In one Canadian study of 789 patients with thrombocytopenia, 204 received second-line therapy for ITP.²³ The relapse rate after the second-line therapy depends on the used modality of treatment. For example, in one study of 137 patients with chronic ITP, 32% of patients who were given rituximab achieved a complete remission that was sustained for 1 year, and 63% were still in remission after 5 years. Two-thirds to 80% of ITP patients remain in remission after splenectomy.²²

There is no one clear criteria to predict if the patient with ITP is going to have a relapse, or how many relapses he is expected to have in a lifetime, but the temporal relationship between the COVID-19 vaccine and the ITP relapses in our

study, and the observation that a significant number of patient developed a relapse within a year following the vaccine regardless of their treatment modality, or time of ITP diagnosis, suggest a causal relationship between mRNA COVID-19 vaccine and ITP relapse.

Based on the reported cases and the conducted observational studies, the current recommendations encourage ITP patients to receive the mRNA COVID-19 vaccine, given the low risk for relapse, and the complete response to the standard ITP treatment.^{2,14} For patients who are having ITP relapse, consideration of delaying the vaccine for patients in active ITP flare till further stabilizations is recommended.²⁴

We believe in the importance of the COVID-19 vaccine in decreasing the number and severity of COVID-19 infection cases, which by far outweigh the risks for the general population, nevertheless, ITP patients might be the group of patients that deserve more discussion about the possibility of relapse after receiving the vaccine, with recommendations for more frequent follow-ups in an extended period for about a year following the COVID-19 vaccine.

Conclusion

mRNA COVID-19 vaccines increase the risk of ITP relapse and can lead to a prolonged reduction in platelet count in a proportion of ITP patients, with no statically significant difference between Comirnaty and Spikevax vaccines. We recommend more frequent follow-up for patients with ITP for 1 year following the mRNA COVID-19 vaccines for early detection and treatment of any significant platelet drop.

Study Limitations

This is a relatively small sample size, and identifying risk factors of ITP relapse in ITP patients and detecting the difference between Spikevax and Comirnaty vaccines needs a larger sample size. There is no control group, and the correlation depends on the temporal relationship between the vaccine and the ITP relapse. A longer follow-up duration to determine the vaccine's longer-term effect on platelet count is needed. Measuring anti-platelet antibodies before and after receiving the vaccine and bone marrow biopsy in resistant cases might help in determining the pathophysiology of patients who become refractory to treatment after receiving the vaccine.

Ethics Statement

MRC/Hamad Medical Corporation ethical committee has reviewed and permitted the study, MRC No 01-21-982. Informed consent was obtained from the study participants. The study has followed the guidelines outlined in the Declaration of Helsinki.

Acknowledgments

Qatar National Library for scientific support.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Vaillant AAJ, Gupta N. ITP-immune thrombocytopenic purpura. StatPearls; 2022; Available from: <https://www.ncbi.nlm.nih.gov/books/NBK537240/>. Accessed March 31, 2023.
2. Immune thrombocytopenia (ITP): practice essentials, background, pathophysiology; 2021. Available from: <https://emedicine.medscape.com/article/202158-overview>. Accessed March 31, 2023.
3. Chen Y, Xu Z, Wang P, et al. New-onset autoimmune phenomena post-COVID-19 vaccination. *Immunology*. 2022;165(4):386–401. doi:10.1111/imm.13443
4. Jawed M, Khalid A, Rubin M, Shafiq R, Cemalovic N. Acute immune thrombocytopenia (ITP) following COVID-19 vaccination in a patient with previously stable ITP. *Open Forum Infect Dis*. 2021;8(7). doi:10.1093/ofid/ofab343
5. Qasim H, Ali E, Yassin MA. Immune thrombocytopenia relapse post covid-19 vaccine in young male patient. *IDCases*. 2021;26:e01344. doi:10.1016/j.idcr.2021.e01344

6. Kuter DJ. Exacerbation of immune thrombocytopenia following COVID-19 vaccination. *Br J Haematol.* 2021;195(3):365–370. doi:10.1111/bjh.17645
7. van Dijk Roger M, Schutgens WE, Roger Schutgens CE, Schutgens RE. Relapse of immune thrombocytopenia after COVID-19 vaccination. *Eur J Haematol.* 2022;108(1):84–85. doi:10.1111/ejh.13713
8. Corbett KS, Flynn B, Foulds KE, et al. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *N Engl J Med.* 2020;383(16):1544–1555. doi:10.1056/NEJMoa2024671
9. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature.* 2020;586(7830):589–593. doi:10.1038/s41586-020-2639-4
10. Chagla Z. In high-risk adults, the Moderna vaccine had 94% efficacy against COVID-19 ≥ 14 d after the 2nd dose. *Ann Intern Med.* 2021;174(3):JC28–9. doi:10.7326/ACPJ202103160-028
11. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med.* 2020;383(27):2603–2615. doi:10.1056/NEJMoa2034577
12. Jara LJ, Vera-Lastra O, Mahroum N, Pineda C, Shoenfeld Y. Autoimmune post-COVID vaccine syndromes: does the spectrum of autoimmune/inflammatory syndrome expand? *Clin Rheumatol.* 2022;41(5):1603. doi:10.1007/s10067-022-06149-4
13. Fragkou PC, Dimopoulou D. Serious complications of COVID-19 vaccines: a mini-review. *Metabol Open.* 2021;12:100145. doi:10.1016/j.metop.2021.100145
14. Lee EJ, Beltrami-Moreira M, Al-Samkari H, et al. SARS-CoV-2 vaccination and ITP in patients with de novo or preexisting ITP. *Blood.* 2022;139(10):1564. doi:10.1182/blood.2021013411
15. Perricone C, Ceccarelli F, Neshet G, et al. Immune thrombocytopenic purpura (ITP) associated with vaccinations: a review of reported cases. *Immunol Res.* 2014;60(2–3):226–235. doi:10.1007/s12026-014-8597-x
16. France EK, Glanz J, Xu S, et al. Risk of immune thrombocytopenic purpura after measles-mumps-rubella immunization in children. *Pediatrics.* 2008;121(3):e687–e692. doi:10.1542/peds.2007-1578
17. Schmidt N, Maitland H. Acute immune thrombocytopenia following administration of Shingrix recombinant zoster vaccine. *Am J Hematol.* 2021;96(5):E136–7. doi:10.1002/ajh.26143
18. Lee EJ, Cines DB, Gernsheimer T, et al. Thrombocytopenia following Pfizer and Moderna SARS-CoV-2 vaccination. *Am J Hematol.* 2021;96(5):534–537. doi:10.1002/ajh.26132
19. Oski FA, Naiman JL. Effect of live measles vaccine on the platelet count. *N Engl J Med.* 2009;275(7):352–356. doi:10.1056/NEJM196608182750703
20. Helms JM, Ansteatt KT, Roberts JC, et al. Severe, refractory immune thrombocytopenia occurring after SARS-CoV-2 vaccine. *J Blood Med.* 2021;12:221. doi:10.2147/JBM.S307047
21. Visser C, Swinkels M, van Werkhoven ED, et al. COVID-19 vaccination in patients with immune thrombocytopenia. *Blood Adv.* 2022;6(6):1637–1644. doi:10.1182/bloodadvances.2021006379
22. McCrae K. Immune thrombocytopenia: no longer ‘idiopathic’. *Cleve Clin J Med.* 2011;78(6):358. doi:10.3949/ccjm.78gr.10005
23. Real world. Available from: <https://www.sciencedirect.com/science/article/pii/S0006497118600497>. Accessed March 31, 2023.
24. Ali E, Al-maharmeh Q, Rozi WM, Habib MB, Yassin M. Immune thrombocytopenia purpura flare post COVID-19 vaccine. *Ann Med Surg.* 2022;75:103164. doi:10.1016/j.amsu.2021.103164

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Military Blood Service in Poland

Tomasz Kryczka, Anna Jedynak, Adam Olszewski, Lidia Sierpiska, Paula Kuzniarska & Dorota Sulejczak

To cite this article: Tomasz Kryczka, Anna Jedynak, Adam Olszewski, Lidia Sierpiska, Paula Kuzniarska & Dorota Sulejczak (2023) Military Blood Service in Poland, Journal of Blood Medicine, , 309-316, DOI: [10.2147/JBM.S390673](https://doi.org/10.2147/JBM.S390673)

To link to this article: <https://doi.org/10.2147/JBM.S390673>



© 2023 Kryczka et al.



Published online: 17 Apr 2023.



Submit your article to this journal



Article views: 385



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Military Blood Service in Poland

Tomasz Kryczka^{1,*}, Anna Jedynak^{2,3,*}, Adam Olszewski^{3,4}, Lidia Sierpiska⁵,
Paula Kuzniarska⁶, Dorota Sulejczak⁷

¹Department of Development of Nursing and Social & Medical Sciences, Medical University of Warsaw, Warsaw, Poland; ²Department of Military Medical Service, Ministry of National Defence of Poland, Warsaw, Poland; ³Military Center of Blood Donation and Haemotherapy, Warsaw, Poland; ⁴Garrison Medical Outpatient Clinic, Modlin, Poland; ⁵Independent Public Health Care Unit, Military Teaching Hospital with Polyclinic, Lublin, Poland; ⁶Military Center of Blood Donation and Haemotherapy, Gdansk, Poland; ⁷Department of Experimental Pharmacology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

*These authors contributed equally to this work

Correspondence: Tomasz Kryczka, Medical University of Warsaw, Department of Development of Nursing and Social & Medical Sciences, ul. Ciołka 27, Warsaw, 01-445, Poland, Tel +48 604315103, Email kryczka.tomasz@gmail.com

Introduction: On 24 February 2022, the Russia–Ukraine military conflict unfolded just across the eastern border of the European Union. It made everyone realize how important it is to secure blood supplies to health-care units in the event of an armed conflict. This paper presents the principles of functioning of the Military Blood Donation Service and the Military Center for Blood Donation and Hemotherapy in Poland.

Methods: The study used data collected in the “Military Blood Bank” information processing system and data from annual reports (2010–2021) sent to the Minister of Health of the Republic of Poland. The reports concerned, among others: demographic data on donors, reasons of permanent disqualifications, numbers of complete and incomplete donations, etc.

Results: Since 2005, the number of donors registered in military blood donation centers ranged between 15 and 35 thousand/year. The most dramatic declines in donors were observed in 2010 and 2020. Successful donations accounted for more than 98% of all donations/year (except 2015), and their number varied between 20 and 32 thousand/year. Among the blood donors, men always predominated and the dominant age group (except for 2010) was 25–44 years. The reasons for permanent disqualification have varied over time: their proportions decreased for viral hepatitis and cardiovascular disease, and increased for respiratory and endocrine/metabolic diseases. Due to the COVID-19 pandemic in 2020/2021, these proportions have sometimes been reversed.

Discussion: The Military Blood Donation Service has been functioning in Poland for several decades. It is specialized in supplying blood and blood products to the Armed Forces. Unfortunately, it was not possible to refer to the functioning of similar institutions in other countries. Therefore, when evaluating the functioning of Polish military blood donation, we had to rely on numerical values (eg, number of donors/year, donor profile, etc.), which prove a very good organization of blood donation centers. However, it should be noted that, as in other countries, a more active promotion of blood donation in the media is advisable in order to encourage as many young people as possible to donate blood.

Keywords: blood donors, blood donation, military blood donation

Introduction

On 24 February 2022, the Russia–Ukraine military conflict unfolded just across the eastern border of the European Union, in the immediate vicinity of Poland, which borders both countries. According to media reports, this war has shaken the sense of security of most countries in the region and prompted them to review their defences and prepare for an escalation of the conflict, also in the medical context.

Considering the latter issue, a continuous supply of blood and blood products to medical institutions is one of the key elements of health care functioning in times of peace and war. In the European Union (EU) countries, the basic standards and guidelines for blood donation are included in relevant directives and regulations (eg, “Blood Guide” regularly updated by the European Committee for Blood Transfusion).¹ In Poland, these regulations are based on the national law and recommendations of authorised institutions, which, however, cannot be incompatible with EU regulations.^{2–4} Poland

and the majority of EU countries also belong to the North Atlantic Treaty Organisation (NATO), which obliges them to implement regulations ensuring the fulfilment of the alliance commitments, among others, in the aspect of blood donation and military blood supply for the time of “peace” (NATO Medical Doctrine).⁵ Key documents (regulations and guidelines) for the time of “war” are created in a confidential mode, eg, at meetings of the Committee of Chiefs of Military Medical Services in NATO (COMEDS).⁶ Additionally, meetings of experts from NATO member countries in the field of military transfusiology (Military Blood Advisory Team, MBAT) are held twice a year. The MBAT is an advisory body to the NATO Military Health Care Working Group (MHCWG) subordinated to the NATO Steering Committee and COMEDS. COMEDS and its agencies initiate and disseminate common foundations, principles, doctrines, concepts, procedures, programmes and techniques for medical security for NATO member countries. The MHCWG is responsible for the standardisation of procedures for medical supplies in the event of emergencies and peacetime in the aspect of military medicine with the highest possible quality of service and safety.⁶

In Poland, the Military Blood Donation (MBD), which functions similarly to civilian blood donation and haemotherapy units (based on WHO and EU guidelines and recommendations) on an everyday basis, has been operating for several decades. At the same time, MBD is specialized in supplying blood and blood products to the Armed Forces based on NATO regulations,^{5,6} like no other civilian institution in the country. Additionally, the Military Centre for Blood Donation and Haemotherapy (MCBDH), whose “flagship” unit is the Independent Public Health Care Institution based in Warsaw, has been operating in Poland since 2005. It consists of seven Field Stations (in larger cities) and the Medical Team in Warsaw.⁷ In addition to controlling and supervising blood treatment in medical entities of the Ministry of National Defence and giving opinions on legal acts in the field of blood donation and haemotherapy in Poland, MCBDH cooperates with national and international organizations, including partners from NATO member states, performing a number of functions specified in NATO regulations for the times of peace.⁸ However, the main task of MCBDH in times of peace or war is to ensure that military and civilian health-care institutions in Poland are provided with blood and blood products.

The aim of this study is to characterize candidates for blood donors reporting to the MCBDH and the reasons for their permanent disqualification since the inception of this unit in Poland.

Materials and Methods

The study used data collected in the “Military Blood Bank” information processing system and data from annual reports sent to the Minister of Health of the Republic of Poland (through the National Blood Centre in Warsaw) from 2010 to 2021. The annual reports concerned the activities of MCBDH, including field branches, mobile teams, personnel, hospitals, serological laboratories, and contained data on donors (number of potential donors available in the system, who can donate blood or its components, number of registered donors in mobile blood collection points, number of donors who donated blood/its components in a given year) by age and sex, number of permanent disqualifications.^{2,3} The reports also included information on the number of complete and incomplete donations (450 mL and 250 mL, respectively), blood components, the total number of donations, an updated list of hospital blood banks supplied, the total number of unused donations, the number of all components manufactured and distributed to health-care units, the occurrence and prevalence of infectious disease markers in blood donors, the number of products withdrawn, as well as the number of documented serious adverse events and reactions.

The collected data were analysed for the demographic profile of donors, number of donations classified as full and incomplete and reasons for disqualification of donors in the subsequent years of service of the military blood donation in Poland. The data accessed complied with relevant data protection and privacy regulations.

The Ethical Committee of Medical University of Warsaw approved this study (Statement No AKBE/171/2019).

Results

The first 5 years of MCBDH activity were characterized by a relatively high public interest in blood donation, with the number of donors between 27 and 35 thousand people per year (data not shown), while after a temporary sharp decline in donations in 2010–2011, the number of donors stabilized at around 21–23 thousand per year in the following years (Table 1). In 2020 and 2021, the number of donors significantly decreased below 20,000 per year, as did the number of

Table 1 Donations Registered at MCBDH in the Years 2010–2021

Years	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Number of donors	15,289	19,163	21,668	22,546	22,286	23,341	22,773	21,982	22,021	21,574	17,169	19,208
Number of donations	21,064	25,489	28,592	30,930	31,221	32,209	31,804	31,431	31,901	32,129	25,854	29,789
First-time donors* (one-time + multiple)	8624 (56%)	10,333 (54%)	11,850 (55%)	11,983 (53%)	9050 (40%)	9147 (39%)	8042 (35%)	8950 (41%)	10,267 (47%)	9641 (44.5%)	6645 (38.7%)	8314 (43.3%)
Repeat donors** (active + reactivated)	6665 (44%)	8830 (46%)	9818 (45%)	10,563 (47%)	13,236 (59%)	14,194 (61%)	14,731 (65%)	13,032 (59%)	11,754 (53%)	11,933 (55.3%)	10,524 (61.3%)	10,150 (52.8%)
Successful donations*** (full donations)	20,793 (98.7%)	25,059 (98.3%)	28,145 (98.4%)	30,448 (98.4%)	30,848 (98.8%)	31,075 (96.5%)	31,356 (98.6%)	31,036 (98.7%)	31,535 (98.9%)	31,780 (98.9%)	25,621 (99.1%)	29,480 (99%)
Unsuccessful donations****	271 (1.3%)	430 (1.7%)	447 (1.6%)	482 (1.55%)	373 (1.2%)	1134 (3.5%)	448 (1.4%)	395 (1.25%)	366 (1.1%)	349 (1.1%)	202 (0.8%)	282 (0.9%)

Notes: *First-time donor (one-time): a person who donated blood/blood components for the first time and did not do it again; first-time donor (multiple): a person who donated blood/blood components for the first time in a given reporting year and continued donating; **Repeat donor (active): a person who donated blood systematically, at least twice in the last 24 months; repeat donor (reactivated): last time donor appeared to donate was over 24 months prior to donation in study year; ***Successful donation: 450mL $\pm$ 10% of blood volume in a single donation; ****Unsuccessful donation: donated blood volume in a single donation < 450 mL $\pm$ 10%.

donations (below 30,000 per year), with the most dramatic drop observed in 2020 (Table 1). Similarly, there was a dramatic fall in first-time donors to 6645 in 2020, whereas in previous years between 8000 and almost 12,000 new donors were registered (Table 1). In 2010, only 6665 repeated donors were registered, and their number increased each year to 14,731 in 2016; currently, the number of repeated donors is in the range of 10–12 thousand people per year (Table 1). Successful donations (so-called “full donations”) accounted for more than 98% of all donations over the analyzed time period (except in 2015, when the percentage of “failures” was about 3.5%; Table 1), and their number increased from 20,793 in 2010 to more than 31,000 in 2019, before dropping to 25,000–29,000/year in the next 2 years (Table 1).

Men predominated among blood donors throughout the history of MCBDH – there were always 2–3 times more men than women, with almost 4 times more men donating blood than women in 2020 and 2021 (Table 2). Men aged 18–24 years constituted the majority of donors only in 2010. In subsequent years, men aged 25–44 years were the predominant group. The percentage of men donating blood aged 25–44 years increased over the years from 18.6% in 2010 to 54.4% in 2021, similarly to women – from 6% to 12.5%. At the same time, the number of young blood donors aged 18–24 years decreased significantly from 45.3% to 13.3% (men) and 19% to 5.25% (women); see Table 2. Young people and people aged ≥ 65 years accounted for less than 1% of donors over the observed time period.

Table 3 shows the main reasons for disqualification recorded at blood donation centers. Attention is drawn to the significant decrease in the number of disqualifications from 329–500 in 2005–2008 to 85–111 in 2019–2021. The data

Table 2 Age Characteristics of Blood Donors in Selected Years

Years	2010	2014	2018	2019	2020	2021
Number of donors	15,289	22,286	22,021	21,574	17,169	19,208
Male (age):						
18–24	45.30%	26.20%	23%	21.20%	15.70%	13.30%
25–44	18.60%	35.60%	42.60%	45.13%	52.03%	54.40%
45–65	7.30%	6.40%	7.80%	8.70%	10.91%	11.10%
Age <18 or 65+	0.20%	0.10%	0.07%	0.04%	0.02%	0%
Total	71.40%	68.30%	73%	75.07%	78.66%	78.80%
Female (age):						
18–24	19.00%	18.40%	13.50%	10.85%	6.24%	5.25%
25–44	6%	10.80%	11%	11.40%	12.21%	12.50%
45–65	3%	2.24%	2.40%	2.63%	2.84%	3.42%
Age <18 or 65+	0.40%	0.14%	0.10%	0.06%	0.01%	0%
Total	28.40%	31.58%	27.00%	24.94%	21.30%	21.17%

Table 3 Causes of Permanent Disqualification Among Blood Donors

Years	Number	A. Skin Diseases %*	B. Viral Hepatitis %*	C. Pulmonary System Diseases %*	D. Metabolic and Endocrine Diseases %*	E. Circulatory System Diseases %*	Other Reasons** %
2005	329	8.81 (23.95)	15.20 (41.33)	1.52 (4.13)	3.04 (8.27)	8.21 (22.32)	63.22
2006	373	12.87 (28.24)	15.82 (34.71)	5.36 (11.76)	6.17 (13.54)	5.36 (11.76)	54.42
2007	418	11.48 (29.80)	10.29 (26.71)	3.83 (9.94)	6.70 (17.39)	6.22 (16.15)	61.48
2008	500	6.20 (19.50)	11.20 (35.22)	2.80 (8.81)	5.20 (16.35)	6.40 (20.13)	68.20
2009	274	8.39 (33.82)	6.93 (27.93)	3.65 (14.71)	2.19 (8.83)	3.65 (14.71)	75.18
2010	220	9.55 (22.11)	7.73 (17.90)	10.91 (25.26)	10.00 (23.15)	5.00 (11.58)	56.82
2011	254	7.48 (20.21)	3.54 (9.56)	12.60 (34.04)	9.45 (25.53)	3.94 (10.65)	62.99
2012	239	16.32 (30.01)	10.04 (18.46)	15.06 (27.69)	6.28 (11.55)	6.69 (12.30)	45.61
2013	192	14.06 (25.23)	9.38 (10.83)	13.54 (24.30)	10.94 (19.63)	7.81 (14.01)	44.27
2014	155	11.61 (18.94)	6.45 (10.52)	29.68 (48.43)	8.39 (13.69)	5.16 (8.42)	38.31
2015	141	17.02 (28.23)	9.93 (16.47)	21.28 (35.30)	6.38 (10.58)	5.67 (9.41)	39.72
2016	161	18.01 (25.66)	4.35 (6.20)	16.15 (23.01)	26.09 (37.17)	5.59 (7.96)	29.81
2017	151	20.53 (29.81)	4.64 (6.74)	18.54 (26.92)	19.87 (28.85)	5.30 (7.69)	31.13
2018	148	14.19 (17.95)	6.76 (8.55)	16.89 (21.37)	37.16 (47.01)	4.05 (5.12)	20.95
2019	111	10.81 (19.67)	- (-)	14.41 (26.23)	27.93 (50.82)	1.80 (3.28)	45.04
2020	85	11.76 (21.28)	3.53 (6.38)	15.29 (27.66)	18.82 (34.04)	5.88 (10.64)	44.70
2021	111	6.31 (16.67)	0.90 (2.38)	4.50 (11.90)	16.22 (42.86)	9.91 (26.19)	62.16

Notes: *The numbers in brackets apply only to disqualifications due to diseases indicated in columns A-E, and are percentages as well. **Other reasons for disqualification: history of the central nervous system disease, eg epilepsy, mental disorders, recurrent faintings, frequent seizures; chronic or recurrent gastrointestinal system diseases including non-viral liver diseases; acute, chronic or recurrent diseases of the urinary tract; malignant tumors; acute, chronic or recurrent haematological diseases; infectious or parasitic diseases (eg, syphilis, AIDS, babesiosis, Chagas fever, leishmaniasis, actinomycosis, tularemia etc.); abuse of narcotics.

collected show an increase in disqualifications due to respiratory diseases from 1.52% in 2005 (10.91% in 2010) to a maximum of 29.68% in 2014, followed by a decrease to values of a few percent in subsequent years and 4.5% in 2021 (Table 3). Even more dramatic decreases were observed for viral hepatitis, from 15.20% in 2005 to values of a few percent between 2013 and 2021 (no disqualification due to viral hepatitis was registered in 2019, Table 3). Similarly, a reduction in the number of disqualifications, eg, due to cardiovascular diseases, was recorded from 8.21% in 2005 to 1.80% in 2019, although the last 2 years have seen a significant increase in disqualifications for this reason (5.88% and 9.91% of potential donors, respectively, Table 3).

As for the increases in disqualifications, those related to endocrine function and metabolic diseases are noteworthy: from 3.04% in 2005 to 19.87%–37.16% between 2016 and 2018, followed by a moderate decrease in these disqualifications in subsequent years (Table 3). Even more significant are disqualifications for so-called “other reasons” (see legend Table 3), from 63.22% in 2005 to 20.95% in 2018, with an increase in the percentage of this category among potential donors in recent years – up to the value recorded 15 years ago (Table 3).

Due to the significant registration of disqualifications for “other reasons than systemic diseases” indicated in Table 3, data on disqualifications due to (i) cardiovascular-, (ii) pulmonary-, (iii) dermatology-, (iv) infectious liver- and (v) metabolic/endocrine diseases were reanalysed, comparing only these 5 reasons of disqualification. Among these 5 reasons for disqualification, cardiovascular diseases accounted for 22.32% in 2005 and less than 10% between 2014 and 2019 (Table 3). However, there was a significant increase in these disqualifications to 26.19% in 2021. In contrast, the most significant decrease in disqualifications was observed for viral hepatitis, from 41.33% to a few percent between 2016 and 2021. For pulmonary diseases, an increase in disqualifications was observed from 4.13% in 2005 to values 5–10 times higher between 2010 and 2020, and an equally high increase in disqualifications due to metabolic and endocrine diseases (Table 3). For skin diseases, the percentage of disqualifications for this reason was variable in both sets (with and without “other”) over the study period (Table 3).

Discussion

The Military Blood Donation has been functioning in Poland for several decades now. The MCBDH also belongs to the MBD as a relatively new organisational unit, which constitutes a significant supplement to the national blood donation and haemotherapy system.⁸ Maintaining a constant number of donations at the level of several dozen thousand/year for

over 15 years is an indicator of the proper functioning of the military blood donation system (see [Table 1](#)). This is a significant observation as in some European countries, eg, Germany, the Netherlands or Switzerland, the number of blood donations is decreasing year by year.^{9–11} According to Chandler et al, this is related to the takeover of part of the donation market in these countries by private organizations or foundations.¹² In the case of Poland, such a sharp decline in the number of donors was observed in 2020 and 2021, ie, when the threat of the COVID 19 pandemic was widely announced. The imposed restrictions strongly discouraged visits to medical facilities worldwide by healthy people who feared infection with the SARS-COV2 virus.¹³ In Poland, the number of first-time donors dropped at that time ([Table 1](#)), ie, those who did not have direct, practical knowledge about the donation procedure itself and were discouraged to visit the medical institution due to the risk of virus infection.

In our reports conducted since 2005, we observed a decrease in the number of donors aged 18–24 years in favour of an older group (25–44 years, [Table 2](#)). This may have resulted from insufficient knowledge of blood donation among young people, especially considering the fact that some authors indicate insufficient promotion of blood donation in the media, eg, social media.^{14–17} Fear of seeing own blood, fear of the procedure itself and negative experiences from previous, first-time blood donation or lack of appropriate “incentives”, eg, financial, may be an additional barrier for young people.^{17–25} Interestingly, women, who are more altruistic than men and more motivated to donate blood,¹⁹ accounted for only 1/4–1/3 of all donors in our analysis ([Table 3](#)). On the other hand, a similar proportion of female blood donors was also observed in other countries.^{12,26,27} According to Bilal et al, women are more likely to drop out of blood donation when they have limited access to a donation point (distance) or lack encouragement from supervisors at workplaces.²⁸ An additional limitation is the medical regulation that a woman can only donate blood 4 times a year.^{2,3}

With regard to the functioning of the MCBDH, it is noteworthy that the proportion of repeated donors over first-time donors has been increasing over time, and since 2014, repeated donors have outnumbered first-time donors ([Table 1](#)). On the one hand, this observation has positive connotations, implying that the medical organization and the atmosphere at the donation centre have made a positive impression on first-time donors, which, according to Alfieri et al, is important for attempting another donation.¹⁹ On the other hand, it may indicate a moderate or low interest in blood donation, especially among young people, which requires specific actions to promote this activity, as previously indicated, eg, in social media.^{14–17}

In the course of MCBDH activity until 2021, 494,810 blood donations were collected from 420,086 donors, with a total number of disqualifications of 3862 people (data not shown). In the early years after establishing MCBDH, the annual number of disqualifications reached 329–500 donors, and the vast majority of these were defined for reasons “other” than diseases of the skin, respiratory-, circulatory-, metabolic/endocrine systems and viral hepatitis (see [Table 3](#), columns “A–E”). By 2018, there was a decline in such “other” disqualifications to a record low of 20.95% in 2018, but in subsequent years it was 44–62% ([Table 3](#)). Disqualifications for “other reasons”, in a range that is very broad in terms of medical interpretation (see legend of [Table 3](#)), dropped by 2018 in favour of, for example, pulmonary diseases. It should be presumed that increasing environmental and especially air pollution has led to an increase in the incidence of lung diseases in recent years,²⁹ which may have gone undiagnosed due to insufficient availability of prevention and treatment of these conditions in Poland.³⁰ Therefore, it should be assumed that it was only during the qualification for blood donation that candidates for donors found out about their disease, hence the multiple increase in disqualifications for this reason comparing successive years of MCBDH activity ([Table 3](#)).

Surprisingly, there was a decrease in the number of disqualifications due to cardiovascular diseases, more noticeable compared to the main reasons for disqualification ([Table 3](#), “A–E”), in the context of persistently high incidence and death rates due to these diseases.^{31,32} It is possible that awareness of one’s own cardiovascular health problems or treatment used³³ significantly influence these patients’ decisions to donate blood, hence the decrease in such disqualifications ([Table 3](#)). What is equally positively surprising is the decrease in disqualifications of donors due to viral hepatitis ([Table 3](#)). At the beginning of the MCBDH activity, hepatitis B or C virus infections were the most common reasons for disqualification (apart from “other reasons”). Currently, in percentage terms, the values are in single digits ([Table 3](#)). The above may be a result of very intensive information and prophylactic campaigns conducted in Poland aimed at this group of diseases over many years, in which emphasis was placed on prevention of infections, in particular through vaccination and educational activities. As a result, there is a noticeable decrease in the incidence of viral hepatitis in Poland,^{34,35} and thus also the likelihood of a patient with undiagnosed hepatotropic virus infection reporting to a blood donation center.

The most significant increase in disqualifications in the observed period of time was reported for metabolic or endocrine diseases, ie, from single to double digit values (Table 3). Among the above diseases, diabetes mellitus came first, as a growing health problem in Poland, but also obesity, and thyroid dysfunction, etc.³⁶ The above is mainly related to changes in nutrition and lifestyle of Poles, which has already been discussed on scientific forums many times. This is not only a problem of Poland, but of the vast majority of industrialized countries, where a sedentary lifestyle and reduced physical activity result in metabolic and endocrine problems of varying severity.³⁷

A certain limitation of the analysis presented in this paper is the fact that most disqualifications recorded at donation points are only in a small part based on the results of laboratory tests – eg, tests for the presence of hepatotropic viruses, HIV, etc. The vast majority of such decisions are based on a questionnaire completed by a potential donor at the initial stage of qualification for donation. Interview with the MD, during which, on the basis of subjective or physical symptoms, the doctor makes an independent decision on permanent or temporary disqualification, is another “screening” stage. During the interview, the doctor is not required to make a diagnosis of the potential donor’s disease due to the limited availability of diagnostic tools, and therefore indicates in general terms the reasons for disqualification in the final report. Due to the prevalence of individual disqualification reasons, we have considered a few as the main ones (see “A-E”, Table 3), and have referred to the remaining disqualification reasons beyond those indicated above as “other reasons”.

The second limitation to this analysis, in which we attempted to reliably assess the functioning of the MCBDH since its inception, was the outbreak of the COVID19 pandemic. At that time, some potential donors declined to donate blood precisely because of the threat associated with the SARS-COV2 virus, which was noticeable in terms of the number of first-time donors. In addition, in the last 2 years, a more self-critical approach to their own health was observed among donors in terms of medical symptoms, such as persistent cough, which may indicate infection with this virus.³⁸ This is likely to be the reason for the sharp decline in disqualifications due to pulmonary diseases in 2021 (Table 3).

The third limitation of this work was the inability to compare the activities of MCBDH in Poland with analogous institutions in other countries due to the lack of literature reports on the subject and the lack of access to data, which, due to the military nature of the units, were not available for us to review. Most of the available publications and reports refer to the functioning of military blood donation, eg, in places of warfare or in the immediate resource base (hospital units, frontline units), possibly in hospitals where military veterans reside. Therefore, our report should be treated as unique on the global scale, in which we indicate the state of functioning of military blood donation in Poland during the period of “peace”, without violating military secrecy concerning actions in the times of “war”.

Conclusions

Considering the current military threats appearing near the eastern border of Poland (conflict between Russia and Ukraine), it should be stated on the basis of the presented analysis, without the possibility of referring to similar institutions in other countries, that the organization and functioning of military blood donation and blood therapy in Poland is excellent, as evidenced by the relatively stable number of donors over the past years despite the changing profile of the donors themselves. However, it should be noted that, as in other countries, a more active promotion of blood donation in the media is advisable in order to encourage as many young people as possible to donate blood.

Abbreviations

MBAT, Military Blood Advisory Team; MHCWG, Military Health Care Working Group; COMEDS, Committee of Chiefs of Military Medical Services in NATO; MBD, Military Blood Donation; MCBDH, Military Centre for Blood Donation and Haemotherapy.

Disclosure

The authors have no relevant or potential conflicts of interest in this work.

References

1. European Directorate for the Quality of Medicines & HealthCare (EDQM). Council of Europe. Guide to the Preparation, Use and Quality Assurance of Blood Components. Recommendation No. R (95) 15; 2020. Available from: www.edqm.eu. Accessed September 6, 2022.

2. State Minister of Health of Republic of Poland. Announcement on the requirements of good practice for: collecting blood and its components, testing, preparation, storage, issuing and transport for organizational units of the public blood service. Official Journal of the Minister of Health 2021, No. 28. Available from: <https://dziennikustaw.gov.pl/>. Accessed September 6, 2022.
3. State Minister of Health of Republic of Poland. Regulation on the conditions for collecting blood from candidates for blood donors and blood donors. Journal of Laws 2017. No 1741. Available from: <https://dziennikustaw.gov.pl/>. Accessed September 6, 2022.
4. Parliament of Republic of Poland. Act on the public blood service; with later changes. Journal of Laws 2021. No 1749. Available from: <https://dziennikustaw.gov.pl/>. Accessed September 6, 2022.
5. North Atlantic Treaty Organization. NATO Standardizations Office (NSO): allied joint doctrine for medical support, C ed., version 1; 2019. Available from: <https://shape.nato.int>. Accessed September 6, 2022.
6. North Atlantic Treaty Organization. Recommendations of the committee of the chiefs of military medical services in NATO; 2021. Available from: <https://www.nato.int/cps/en/natohq/topics-175736.html>. Accessed September 6, 2022.
7. State Minister of National Defense of Republic of Poland. Regulation on the establishment of the military center for blood donation and haemotherapy. Journal of Laws 2004. No 243, Item 2433; 2004. Available from: <https://dziennikustaw.gov.pl/>. Accessed September 6, 2022.
8. Jedynak A, Klamrowski P, Olszewski A, Koper D, Sienkiewicz Z, Kryczka T. Krwiodawstwo i Krwiolecznictwo wojskowe w Polsce [Blood donation and military blood haemotherapy in Poland]. *Surg Vasc Nurs*. 2020;42:64–69. Polish.
9. Volken T, Buser A, Castelli D, et al. Red blood cell use in Switzerland: trends and demographic challenges. *Blood Transfus*. 2018;16(1):73–82. doi:10.2450/2016.0079-16
10. Müller-Steinhardt M, Weidmann C, Klüter H. Changes in the whole blood donor population in South-West Germany: 2010 versus 2016. *Transfus Med Hemother*. 2017;44(4):217–223. doi:10.1159/000479192
11. Borkent-Raven BA, Janssen MP, Van Der Poel CL. Demographic changes and predicting blood supply and demand in the Netherlands. *Transfusion*. 2010;50(11):2455–2460. doi:10.1111/j.1537-2995.2010.02716.x
12. Chandler T, Hiller J, Peine S, Stargardt T. Blood donation and donors: insights from a large German teaching hospital (2008–2017). *Vox Sang*. 2020;115(1):27–35. doi:10.1111/vox.12853
13. Khalilpourazari S, Hashemi Doulabi H. A flexible robust model for blood supply chain network design problem. *Ann Oper Res*. 2022;21:1–26.
14. Beyene GA. Voluntary blood donation knowledge, attitudes, and practices in central Ethiopia. *Int J Gen Med*. 2020;13:67–76. doi:10.2147/IJGM.S246138
15. Al-Hajri QR, Alfayez A, Alsalman D, et al. The impact of WhatsApp on the blood donation process in Saudi Arabia. *J Blood Med*. 2021;12:1003–1010. doi:10.2147/JBM.S339521
16. Ramondt S, Kerkhof P, Merz EM. Blood donation narratives on social media: a topic modeling study. *Transfus Med Rev*. 2022;36(1):58–65. doi:10.1016/j.tmr.2021.10.001
17. Ramondt S, Zijlstra M, Kerkhof P, Merz EM. Barriers to blood donation on social media: an analysis of Facebook and twitter posts. *Transfusion*. 2020;60(10):2294–2306. doi:10.1111/trf.15998
18. Ugwu NI, Uneke CJ, Ugwu CN, Oti WJO, Agbo UN, Akamike IC. Effect of blood donor educational intervention on the knowledge and attitude towards voluntary blood donation among medical students at a Nigerian university. *Niger Med J*. 2020;61(3):163–168. doi:10.4103/nmj.NMJ_177_19
19. Alfieri S, Guidi P, Marta E, Saturni V. Economic crisis and blood donation: how are donors motivations changing? *Transfus Apher Sci*. 2016;54(3):396–400. doi:10.1016/j.transci.2016.03.001
20. Oti HM, Asiri MA, Ahmad MT, et al. Measuring public awareness about blood donation in Assir, South-Western Saudi Arabia. *Transfus Clin Biol*. 2020;27(3):122–127. doi:10.1016/j.trcli.2020.06.005
21. France CR, France JL, Himawan LK, et al. Fear is associated with attrition of first-time whole blood donors: a longitudinal examination of donor confidence and attitude as potential mediators. *Transfusion*. 2021;61(12):3372–3380. doi:10.1111/trf.16671
22. Rael CT, Pierre D, Frye V, et al. Evaluating blood donor experiences and barriers/facilitators to blood donation in the United States using YouTube video content. *Transfusion*. 2021;61(9):2650–2657. doi:10.1111/trf.16568
23. Ugwu NI, Oti WJ, Ugwu CN, Uneke CJ. Voluntary non-remunerated blood donation: awareness, perception, and attitude among potential blood donors in Abakaliki, Nigeria. *Niger J Clin Pract*. 2019;22(11):1509–1515. doi:10.4103/njcp.njcp_159_19
24. Veerus P, Kullaste R, Pungas K, Aavik T, Lang K. How to reverse first time donors to become regular donors? A questionnaire survey in Estonia. *Transfus Clin Biol*. 2017;24(4):404–409. doi:10.1016/j.trcli.2017.06.010
25. Alaskar SA, Alsadhan JA, Alanazi RM, et al. Voluntary blood donation among female health care university students in Saudi Arabia, knowledge and status. *J Family Med Prim Care*. 2021;10(6):2353–2357. doi:10.4103/jfmpe.jfmpe_2182_20
26. Bhuiya MSH, Faiyaz SR, Jaman N, Uddin MJ. Factors influencing voluntary blood donation practice among university students of Bangladesh. *Transfus Apher Sci*. 2022;61(4):103379. doi:10.1016/j.transci.2022.103379
27. Saleh RA, Khalil H, Alsaleh M, et al. Voluntary and non-voluntary blood donations among doctors. *Health Sci Rep*. 2021;4(4):e377. doi:10.1002/hsr2.377
28. Bilal M, Haseeb A, Zahid I, et al. Knowledge, attitudes and perceptions among non blood donor female health care professionals. *Glob J Health Sci*. 2015;8(4):203–211. doi:10.5539/gjhs.v8n4p203
29. Nazar W, Niedoszytko M. Air pollution in Poland: a 2022 narrative review with focus on respiratory diseases. *Int J Environ Res Public Health*. 2022;19(2):895. doi:10.3390/ijerph19020895
30. Supreme Chamber of Control of Republic of Poland. Availability of prevention and treatment of respiratory diseases; 2016. Available from: <https://www.nik.gov.pl/plik/id,11909,vp,14281.pdf>. Accessed September 6, 2022.
31. Czerwinska K, Gac P, Poreba R, Mazur G, Pawlas K. Cardiac and vascular disorders as para-occupational diseases - a Polish perspective. *Ann Agric Environ Med*. 2021;28(2):231–236. doi:10.26444/aaem/122259
32. Main Statistical Office of Republic of Poland. Incidence and mortality from cardiovascular diseases and the demographic situation; 2022. Available from: https://stat.gov.pl/files/gfx/portalinformacyjny/pl/defaultaktualnosci/5468/22/1/1/statystyka_umieralnosci_w_wyniku_chuk.pdf. Accessed September 6, 2022.
33. Jankowski P, Banach M, Malyszko J, et al. May measurement month 2018: an analysis of blood pressure screening campaign results from Poland. *Eur Heart J Suppl*. 2020;22(SupplH):H108–H111. doi:10.1093/eurheartj/suaa041
34. Stawinska-Witoszynska B, Klos J, Moryson W, Wieckowska B. Trends in the incidence of acute hepatitis B in the Polish population and their determinants. *Medicina*. 2021;57(8):738. doi:10.3390/medicina57080738

35. Wojtczak A. Review of „the project KIK/35„ prevention of Hepatitis C Virus (HCV) infections” as an example of integrated public health interventions to reduce blood-borne infections in Poland. *Przegl Epidemiol.* 2017;71(3):283–285.
36. Placzekiewicz-Jankowska E, Czupryniak L, Gajos G, et al. Management of obesity in the times of climate change and COVID-19: an interdisciplinary expert consensus report. *Pol Arch Intern Med.* 2022;132(3):16216. doi:10.20452/pamw.16216
37. Lipinska-Ojrzanowska A, Walusiak-Skorupa J, Ojrzanowski M, Marcinkiewicz A, Plewka M, Wiszniewska M. Evaluation of selected risk factors for cardiovascular diseases and diabetes as a background for the prevention program in occupational healthcare. *Int J Occup Med Environ Health.* 2021;34(3):403–413. doi:10.13075/ijomeh.1896.01531
38. Semczuk-Kaczmarek K, Rys-Czaporowska A, Sierdzinski J, Kaczmarek LD, Szymanski FM, Platek AE. Association between air pollution and COVID-19 mortality and morbidity. *Intern Emerg Med.* 2022;17(2):467–473. doi:10.1007/s11739-021-02834-5

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



D-Dimer Level Associated with Amount of Sinus involvement Using Digital Subtraction Angiography on Cerebral Venous Thrombosis Patients

Lisda Amalia

To cite this article: Lisda Amalia (2023) D-Dimer Level Associated with Amount of Sinus involvement Using Digital Subtraction Angiography on Cerebral Venous Thrombosis Patients, Journal of Blood Medicine, , 303-308, DOI: [10.2147/JBM.S399661](https://doi.org/10.2147/JBM.S399661)

To link to this article: <https://doi.org/10.2147/JBM.S399661>



© 2023 Amalia.



Published online: 17 Apr 2023.



Submit your article to this journal



Article views: 281



View related articles



View Crossmark data



Citing articles: 2 View citing articles

D-Dimer Level Associated with Amount of Sinus involvement Using Digital Subtraction Angiography on Cerebral Venous Thrombosis Patients

Lisda Amalia 

Department of Neurology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

Correspondence: Lisda Amalia, Department of Neurology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Jl. Pasteur 38, Bandung, 40161, Indonesia, Email dr.lisda@gmail.com

Background: Cerebral venous sinus thrombosis (CVST) is a form of the cerebrovascular disease characterized by varying degrees of obstruction of veins and cerebral sinuses caused by thrombus. Diagnosis is incredibly challenging due to the wide variety of symptoms and the distinct radiological aspects of affected vessels. In patients with venous thrombosis, the presence of D-Dimer is used as an indicator of the presence of endogenous fibrinolysis. D-Dimer is a by-product of fibrin polymer fragmentation.

Objective: To investigate the relationship between the level of D-Dimer and the number of sinuses in CVST by Digital Subtraction Angiography (DSA).

Methods: Retrospective data from September 2021 to September 2022 were used in this analytical observational with a cross-sectional study design. Chi-Square is used for data processing relationship analysis.

Results: Out of the 54 subjects with elevated levels of D-Dimer, 38 (70.4%) are females, whereas 16 (29.6%) are males. High levels of D-Dimer have been related to a greater risk of sinus thrombosis ($p < 0.001$). D-Dimer levels were similarly shown to rise in conjunction with the number of sinuses most severely damaged by thrombosis. The most common site for thrombosis to develop in this study were the left sigmoid and left transverse sinuses. Most risk factors were hormonal.

Conclusion: There is a statistically significant relationship between an increase in the D-Dimer level and the greater number of sinuses involved as determined by DSA in individuals diagnosed with CVST.

Keywords: cerebral venous sinus thrombosis, D-Dimer, sinus involvement

Introduction

Cerebral Venous Sinus Thrombosis, also known as CVST, is a form of cerebrovascular disease that manifests as a narrowing to occlusion in the vein channels and cerebral sinuses resulting from a thrombus. This condition, which may display a wide range of clinical symptoms and radiological findings, is very challenging to identify. Serious consequences, including infarction, hemorrhage, and death, may result from misdiagnosing this condition.¹⁻³ The yearly incidence rate was predicted to be 1.32 cases per 100.000 people in 2005, but it has since increased to 2.62 cases per 100.000 population.⁴⁻⁶ With 61% of females between the ages of 20 and 35 affected, the female's incidence rate is much greater than that of males. The female-to-male ratio is 3:1.^{4,5} This pertains to pregnancy or the usage of contraceptive pills. The research by Lisda Amalia at Hasan Sadikin General Hospital Bandung discovered that the largest incidence of CVST was in women (81%) with an average age of 37.68 years. This result was in accordance obtained with the findings of the study.⁷

In individuals with venous thrombosis, the presence of D-Dimer may be used as an indicator of endogenous fibrinolysis. Cross-linked fibrin fragments dissolved by plasmin, known as D-Dimers, have been demonstrated to aid in diagnosing venous thrombosis by measuring plasma levels. D-Dimer has a high sensitivity, but its specificity is poor. From the onset of symptoms until the subacute or chronic phase, D-Dimer levels might decline over time.^{3,4} The

structure of a thrombosed sinus has reported to play a role in the D-Dimer findings, and this might be another reason for a drop in D-Dimer levels.

Non-thrombotic pathological diseases, such as cancer, liver disease, renal disease, organ-graft healing, and thrombolysis therapy, may also produce significant levels of D-Dimer.^{8–10} The number of cerebral venous sinuses implicated was shown to have a substantial relationship with D-Dimer levels, with higher D-Dimer levels corresponding to a larger number of cerebral venous sinuses.^{11–14} The rise in D-Dimer levels has been linked to the development of thrombosis in numerous cerebral venous sinuses and to the hypothesis positing that such blockages account for the vast majority of cases with CVST.^{15,16} The greater the number and severity of blockages in CVST cases, the greater the formation of fibrin polymers, which are subsequently degraded into D-Dimer.^{11–13}

Digital Subtraction Angiography has a higher sensitivity (95%), and specificity (91%), respectively, than both Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans. DSA is thus preferable for evaluating normal anatomy and blockage (occlusion) in the veins.^{16,17} DSA is the gold standard test and should be performed on patients with clinical suspicion of CVST but normal results on CT Venography or Magnetic Resonance Venography (MRV).^{18–23} This research finding corresponds D-Dimer levels to DSA-measured sinuses in CVST patients.

Methods

This research is an analytical observational study using a retrospective cohort design, and it was conducted on 54 participants who were diagnosed with CVST between September 2021 and September 2022. The patient's medical records were retrieved in compliance with the hospital's ethical guidelines by maintaining confidentiality. Patients diagnosed with CVST had their demographic information, clinical features, risk factors, D-Dimer levels, and DSA (imaging) results defined. History of deep vein thrombosis and pulmonary embolism were excluded in this study. Chi-Square is used for data processing relationship analysis. All patients gave their informed permission in writing. This study complied with all relevant ethical regulations (including The Declaration of Helsinki) and covering patient data confidentiality. The correct ethics procedure was followed. Ethical clearance for the study (LB.02.01/X.6.5/347/2022) was granted by the research ethics committee at Hasan Sadikin General Hospital in Bandung.

Results

Clinical Characteristics of Study Participants

The sample size of this research included 54 CVST patients ([Supplementary Material Table 1](#)), with a mean age of 44.31 (ranging from 29 to 70 years old) and a much higher proportion of female patients than male patients (70.4%). [Table 1](#) shows that Diabetes Mellitus (DM) and dyslipidemia are the most prevalent cause of sinus thrombosis, and the most common sites affected by thrombosis are the left transverse and sigmoid sinuses.

High D-Dimer Levels and Sinus Distribution

[Table 2](#) illustrates the relationship between elevated D-Dimer levels, the amount, and the location of sinuses subjects. D-Dimer levels rise in proportion to the number of sinuses. $P < 0.001$ ($p\text{-value} \leq 0.05$) indicates statistical significance.

Increased D-Dimer and Sinuses

[Table 3](#) displays the correlation between the location of the sinuses and elevated D-Dimer levels in individuals with CVST who underwent DSA. [Table 3](#) presents that the left transverse sinus and left sigmoid are the most common sites of sinus thrombosis. The relationship between sinus location and elevated D-Dimer levels is statistically significant ($p < 0.001$).

Factors Associated with Elevated D-Dimer Levels

[Table 4](#) depicts the relationship between risk factors and elevated D-Dimer levels in CVST patients who underwent DSA. The table indicates a significant relationship between elevated D-Dimer levels and risk factors at the $p\text{-value} 0.035$ ($p\text{-value} \leq 0.05$). Hormonal is the most prevalent risk factor identified in this study, affecting 14 subjects.

Table 1 Characteristic of Research Subjects

Variable	n	%	Mean(SD)
Age (year)			44.31 (12.347)
20–30 year	5	9.3	
31–40 year	18	33.3	
41–50 year	15	27.8	
51–60 year	8	16.7	
61–70 year	7	13.0	
Gender			
Male	16	29.6	
Female	38	70.4	
Amount of sinus involvement			
Single	34	63.0	
Multiple	20	37.0	
Sinus location			
Right Sinus Transversus	5	9.3	
Left Sinus Transversus	14	25.9	
Bilateral Transversus	1	1.9	
Sinus Sagitalis superior	1	1.9	
Left Sinus Transversus and sigmoid	26	48.1	
Bilateral Sinus Transversus and sigmoid	5	9.3	
Left Sinus Transversus and sagitalis superior	1	1.9	
Left Sinus Transversus and straight sinus	1	1.9	
Risk factors			
Autoimmune	1	1.9	
Hormonal	16	29.6	
Malignancy	2	3.7	
Hematological disorders	13	24.1	
Diabetes Mellitus/dyslipidemia	22	40.7	

Table 2 Relationship Between D-Dimer Level and Amount of Sinus Involvement

Variable	D-Dimer Level				Total		p value
	High Level		Normal				
	n	%	n	%	n	%	
Amount of sinus							<0.001*
Single	3	15	17	85	24	100.0	
Multiple	32	94.1	2	5.9	34	100.0	

Note: *Statistically significant.

Discussion

Patients with CVST diagnosed between September 2021 and September 2022 at Hasan Sadikin Bandung General Hospital are profiled in this study, including their ages, sexes, D-Dimer levels, and the number of affected sinuses. The proportion of the research participants in this study was more females than males, with an average age distribution of 44.31 years (Table 1). This feature is consistent with the finding that CVST is more common in females, as reported in prior research.^{7,16}

Antiphospholipid syndrome, genetic susceptibility, and other acquired prothrombotic disorders such as pregnancy, postpartum period, contraceptive pills, malignancy, infection, and trauma are major risk factors for developing CVST.^{24–28} The pathogenesis of cerebral venous sinus thrombosis may be inferred to include two distinct processes. Intracranial

Table 3 Relationship Between D-Dimer Level and Sinus Location Involvement

Variable	D-Dimer Level				Total		p-value
	High		Normal				
	n	%	n	%	n	%	
Sinus location							<0.001*
Right Sinus Transversus	0	0.0	5	100.0	5	100.0	
Left Sinus Transversus	3	21.4	11	78.6	14	100.0	
Bilateral Sinus Transversus	1	100.0	0	0.0	1	100.0	
Sinus Sagitalis superior	0	0.0	1	100.0	1	100.0	
Left Sinus Transversus and sigmoid	25	96.2	1	3.8	26	100.0	
Right Sinus Transversus and sigmoid	5	100.0	0	0.0	5	100.0	
Left Sinus Transversus and sagitalis superior	1	100.0	0	0.0	1	100.0	
Left Sinus transversus and straight sinus	0	0.0	1	100.0	1	100.0	

Note: *Statistically significant.

Table 4 Relationship Between D-Dimer Level and Risk Factors

Variable	D-Dimer Level				Total	p-value
	High		Normal/Low			
	n	%	n	%		
Risk factors						0.044*
Autoimmune	1	100	0	0	1	
Hormonal	14	87.5	2	12.5	16	
Malignancy	2	66.7	1	33.3	3	
Hematology disorders	8	53.3	7	46.7	15	
Diabetes Mellitus/ Dyslipidemia	10	43.5	13	56.5	23	

Note: *Statistically significant.

pressure increases in the case of cerebral sinus thrombosis, while localized effects in the case of cerebral venous thrombosis owing to venous blockage. The vast majority of patients can go through both of these mechanisms simultaneously.^{23–34} Elevated D-Dimer levels are often used as a standard for measuring the coagulation process. However, medical and physical conditions may affect plasma D-Dimer levels. D-Dimer levels were elevated in individuals suffering from pulmonary embolism, deep vein thrombosis (DVT), and disseminated intravascular coagulation (DIC).³⁴

Additionally, patients with infections and after major surgery often experienced elevated D-Dimer levels because of the accelerated breakdown of extravascular fibrin.^{28–33} In this study, hormonal usage in women between the ages of 20 and 35 was the risk factor related to elevated D-Dimer levels (Table 4).^{7,35} This confirms the findings of the previous research, which found that hormonal imbalance is a significant risk factor in CVST patients.^{6,7,36}

Diagnosing CVST is complicated by patients' often-atypical symptoms, a wide range of risk factors, and thrombus often develops in the brain's veins and sinuses.^{4,5,23–32} DSA is a gold standard for identifying vascular diseases like thrombosis, aneurysms, and ulcerative plaques. DSA is essential to determine the diagnosis of CVST.^{7,34,35,37–43} CVST causes 1–2% of adult strokes and affects all ages. CVST is more frequently observed in the transverse sinuses (62%).¹⁰ In this study, the most prevalent sites for thrombosis were the left transverse sinus and left sigmoid sinus (Table 3).

D-Dimer elevations in CVST patients were observed to be strongly associated with the number of sinus involvement (Table 2).^{17,44–48} Multiple sinuses are impacted when the D-Dimer level is higher because additional thrombus formation circumstances arise.^{6,47,49–54} D-Dimer levels were positively correlated with thrombus extension, with high levels in the presence of larger thrombi. D-Dimer levels had an inverse relation with a duration between the onset of symptoms and testing, typically reaching 25% of the initial value after 1–2 weeks.⁵⁵

Limitation of Study

1. Since the data is retrospective, patients could not be followed up after DSA.
2. Without a comparison group, it is impossible to determine if asymptomatic individuals exhibit any structural abnormalities in their blood vessels when exposed to CVST.
3. Neither DSA nor any similar follow-ups were attempted.

Conclusion

There is a significant relationship between the D-Dimer level and the number of sinus involvement in CVST patients utilizing DSA, which often affects the left transverse sinus and left sigmoid.

Funding

No funding received for this study.

Disclosure

The author reports no conflicts of interest in this work.

References

1. Tatlisumak T, Jood K, Putaala JJS. Cerebral venous thrombosis: epidemiology in change. *Stroke*. 2016;47(9):2169–2170. doi:10.1161/STROKEAHA.116.014336
2. Chiewvit P, Piyapittayan S, Pongvarin N. Cerebral venous thrombosis: diagnosis dilemma. *Neurol Int*. 2011;3(3):13. doi:10.4081/ni.2011.e13
3. Saposnik G, Barinagarrementeria F, Brown RD. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42(4):1158–1192. doi:10.1161/STR.0b013e31820a8364
4. Leach JL, Fortuna RB, Jones BV, Gaskill-Shipley MF. Imaging of cerebral venous thrombosis: current techniques, spectrum of findings, and diagnostic pitfalls. *Radiographics*. 2006;26(1):S19–41. doi:10.1148/rg.26si055174
5. Atanassova PA, Massaldjieva RI, Chalakova NT, Dimitrov BD. Cerebral venous sinus thrombosis - diagnostic strategies and prognostic models: a review; 2019. Available from: https://repository.rcsi.com/articles/chapter/Cerebral_Venous_Sinus_Thrombosis_-_Diagnostic_Strategies_and_Prognostic_Models_A_Review/10778111. Accessed April 12, 2023.
6. Ikejiri M, Shindo A, Li Y, et al. Frequent association of thrombophilia in cerebral venous sinus thrombosis. *Japan Soc Hematol*. 2012;95:257–262.
7. Amalia L. Karakteristik klinis pasien trombosis vena serebral (TSVS) di Ruang Rawat Inap Neurologi Rumah Sakit Hasan Sadikin Bandung [Clinical Characteristics of Cerebral Venous Sinus Thrombosis (CVST) Patients in Neurology Ward Hasan Sadikin General Hospital Bandung]. *J Neuro Anestesi Indo*. Indonesian. 2020;2020(2):71–77.
8. Piazza G. Cerebral venous thrombosis. *Circulation*. 2012;125(13):1704–1709. doi:10.1161/CIRCULATIONAHA.111.067835
9. Ibrahim EAA, Mohamed EH, Ahmed KA, Omer MEA. Clinical profile and risk factors of cerebral venous sinus thrombosis (CVST) in Sudan. *Res Square*. 2021;1:1–16.
10. Putra AA, Buchori E, Hilman H, Amalia L. D-dimer level with cerebral venous sinus thrombosis occurrence using digital subtraction angiography: global medical and health communication. *Neurosurg Focus*. 2019. doi:10.29313/gmh.v7i3.5341
11. Crassard I, Soria C, Tzourio C. A negative D-dimer assay does not rule out cerebral venous thrombosis: a series of seventy-three patients. *Stroke*. 2005;36(8):1716–1719. doi:10.1161/01.STR.0000173401.76085.98
12. Raskob GE, Angchaisuksiri P, Blanco AN. Thrombosis: a major contributor to global disease burden. *Arterioscler Thromb Vasc Biol*. 2014;34(11):2363–2371. doi:10.1161/ATVBAHA.114.304488
13. López JA, Chen J. Pathophysiology of venous thrombosis. *Thromb Res*. 2009;123(4):30–34. doi:10.1016/S0049-3848(09)70140-9
14. Ferro JM, Aguiar de Sousa D. Cerebral venous thrombosis: an update. *Curr Neurol Neurosci Rep*. 2019;19(10):1–9. doi:10.1007/s11910-019-0988-x
15. Ji EC, Young CW, Park GM. Comparison of MRI sequences for the detection of cerebral venous sinus thrombosis during follow-up examination. *J Korean Soc Radiol*. 2018;78(5):330–339. doi:10.3348/jksr.2018.78.5.330
16. Aditya K, Andhitara Y, Tugasworo D, et al. The correlation of D-dimer level and number of venous sinus in patient with cerebral. *Neurona*. 2020;38(1):1–7.
17. Wetzel SG, Kirsch E, Stock KW, Kolbe M, Kaim A, Radue EW. Cerebral veins: comparative study of CT venography with intraarterial digital subtraction angiography. *Am J Neuroradiol*. 1999;20:249–255.
18. Uflacker RJB. *Atlas of Vascular Anatomy. An Angiographic Approach*. 1st ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
19. Geyer JD, Gomez CR. *Stroke: A Practical Approach*. 1st ed. Philadelphia: Lippincott Williams & Wilkins; 2009.
20. Ferroni P, Martini F, Riondino S. Soluble P-selectin as a marker of in vivo platelet activation. *Clin Chim Acta*. 2009;399(1–2):88–91. doi:10.1016/j.cca.2008.09.018
21. Snell RS, Tambajong J. *Anatomi Klinik: Untuk Mahasiswa Kedokteran* [Clinical anatomy: for medical students]. Jakarta: Penerbit Buku Kedokteran. EGC; Indonesian; 1992.
22. Baehr M, Frotscher MJ. 2010 D. *Diagnosis Topik Neurologi DUUS Anatomi. Fisiologi. Tanda. Gejala* [DUUS Neurology Diagnostics Topics (Anatomy, Physiology, Signs, Symptoms)]. 1st ed. Jakarta: ECG; 2010.
23. Stam J. Thrombosis of the cerebral veins and sinuses. *N Eng J Med*. 2005;352(17):1791–1798. doi:10.1056/NEJMr042354
24. Jellinger KA. Handbook of cerebral venous thrombosis. *Eur J Neurol*. 2009;16(2):e38. doi:10.1111/j.1468-1331.2008.02469.x
25. Zia A, Wasay M, Kaul S. Epidemiology of cerebral venous thrombosis in Asian countries. *Pak J Neurol Sci*. 2014;9(3):43–47.

26. Saliba ZS, Slaba SG, Sawan EB. Hemostasis. In: Butera G, Chessa M, Eicken A, Thomson J, editors. *Cardiac Catheterization for Congenital Heart Disease*. 1st ed. London: Springer; 2015:181–192.
27. Perry DJ. *Hemostasis and Thrombosis Protocols*. 1st ed. New Jersey: Humana Press; 2019.
28. Meng R, Wang X, Hussain M. Evaluation of plasma D-dimer plus fibrinogen in predicting acute CVST. *Int J Stroke*. 2014;9(2):166–173. doi:10.1111/ijss.12034
29. Gale AJ. Continuing education course #2: current understanding of hemostasis. *Toxicol Pathol*. 2011;39(1):273–280. doi:10.1177/0192623310389474
30. Fitridge R, Thompson M. *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists*. 1st ed. Adelaide: University of Adelaide Press; 2011.
31. Colvin B. Essential haematology. *J Clin Pathol*. 1993;46:687–688. doi:10.1136/jcp.46.7.687-e
32. Sevt D, Sahin S, Duman T, et al. Cerebral Venous Sinus Thrombosis in Femoral Veins. *J Clin Pathol*. 1974;27(7):517–528. doi:10.1136/jcp.27.7.517
33. De Freitas GR, Bogousslavsky J. Primary stroke prevention. *Eur J Neurol*. 2001;8(1):1–15. doi:10.1046/j.1468-1331.2001.00150.x
34. Misra U, Kalita J, Bansal V. D-dimer is useful in the diagnosis of cortical venous sinus thrombosis. *Neurol India*. 2009;57(1):50–54. doi:10.4103/0028-3886.48822
35. Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Female hormones and thrombosis. *Am Heart Assoc*. 2002;22(2):201–210.
36. Uluduz D, Sahin S, Duman T, et al. *Cerebral Venous Sinus Thrombosis in Women: Subgroup Analysis of the Venost Study*. Hindawi; 2020.
37. Hashami L, Rakhshan V, Karimian H, Moghaddasi M. Diagnostic value of D-dimer's serum level in Iranian patients with cerebral venous thrombosis. *Neurol Int*. 2016;15(8):6310.
38. Wang J, Ning R, Wang Y. Plasma D-dimer level. the promising prognostic biomarker for the acute cerebral infarction patients. *J Stroke Cerebrovasc Dis*. 2016;25(8):2011–2015. doi:10.1016/j.jstrokecerebrovasdis.2015.12.031
39. Andreescu A, Cushman M, Rosendaal F. D-dimer as a risk factor for deep vein thrombosis: the Leiden Thrombophilia Study. *Thromb Haemost*. 2002;87(1):47–51. doi:10.1055/s-0037-1612942
40. Capecchi M, Abbattista M, Martinelli I. Cerebral venous sinus thrombosis. *J Thromb Haemost*. 2018;16(10):1918–1931. doi:10.1111/jth.14210
41. Tichelaar YGV, Kluin-Nelemans HJC, Meijer K. Infections and inflammatory diseases as risk factors for venous thrombosis. A systematic review. *Thromb Haemost*. 2012;107(05):827–837. doi:10.1160/TH11-09-0611
42. Alvis-Miranda HR, Castellar-Leones SM, Alcalá-Cerra G, Moscote-Salazar LR. Cerebral sinus venous thrombosis. *J Neurosci Rural Pract*. 2013;4(4):427–438. doi:10.4103/0976-3147.120236
43. Canedo-Antelo M, Baleato-González S, Mosqueira AJ. Radiologic clues to cerebral venous thrombosis. *Radiographics*. 2019;39(6):1611–1628. doi:10.1148/rg.2019190015
44. Sherif FM, Belal TME. Contrast-enhanced cerebral MR venography overcomes causes of drop of signal in time of flight MR venography. *Med J Cairo Univ*. 2018;86(8):4385–4390. doi:10.21608/mjcu.2018.62826
45. Weimar C. Diagnosis and treatment of cerebral venous and sinus thrombosis. *Curr Neurol Neurosci Rep*. 2014;14(1):417. doi:10.1007/s11910-013-0417-5
46. Wang HF, Pu CQ, Yin X. D-dimers (DD) in CVST. *Int J Neurosci*. 2017;127(6):524–530. doi:10.1080/00207454.2016.1207172
47. Azeemuddin WM, Azeemuddin M. Neuroimaging of cerebral venous thrombosis. *J Neuroimaging*. 2005;15(2):118–128. doi:10.1111/j.1552-6569.2005.tb00296.x
48. Darmawan G, Hamijoyo L, Oehadian A, Bandiara R, Amalia L. Cerebral venous sinus thrombosis in systemic lupus erythematosus; department of internal medicine. *Acta Med Indones*. 2018;50(4):343–345.
49. Lubicz B, Neugroschl C, Collignon L, François O, Balériaux D. Is digital subtraction angiography still needed for the follow-up of intracranial aneurysms treated by embolisation with detachable coils? *Neuroradiology*. 2008;50(10):841–848. doi:10.1007/s00234-008-0450-2
50. Shankar JJS, Chakraborty LC, Dos Santos MP, Dos Santos MP. Cerebral vascular malformations: time-resolved CT angiography compared to DSA. *Neuroradiol J*. 2015;28(3):310–315. doi:10.1177/1971400915589682
51. Yang QH, Yang M. Early imaging characteristics of 62 cases of cerebral venous sinus thrombosis. *Exp Ther Med*. 2013;5(1):233–236. doi:10.3892/etm.2012.796
52. Saadatnia M, Fatehi F, Basiri K, Mousavi SA, Mehr GK. Cerebral venous sinus thrombosis risk factors. *Int J Stroke*. 2009;4(2):111–123. doi:10.1111/j.1747-4949.2009.00260.x
53. Gijn J. Cerebral venous thrombosis: pathogenesis. presentation and prognosis. *J R Soc Med*. 2000;93(5):230–233. doi:10.1177/014107680009300504
54. Fairbanks A, Chodnicki K, Lesser E, et al. Population-based incidence and visual outcomes of cerebral venous sinus thrombosis. *Invest Ophthalmol Vis Sci*. 2018;59:2176.
55. Gurram M, Pulivarthi S. Effectiveness of D-dimer as a screening test for venous thromboembolism: an update. *N Am J Med Sci*. 2014;6(10):491–499. doi:10.4103/1947-2714.14327

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



RNAi for the Treatment of People with Hemophilia: Current Evidence and Patient Selection

Sara Boyce & Savita Rangarajan

To cite this article: Sara Boyce & Savita Rangarajan (2023) RNAi for the Treatment of People with Hemophilia: Current Evidence and Patient Selection, Journal of Blood Medicine, , 317-327, DOI: [10.2147/JBM.S390521](https://doi.org/10.2147/JBM.S390521)

To link to this article: <https://doi.org/10.2147/JBM.S390521>



© 2023 Boyce and Rangarajan.



Published online: 22 Apr 2023.



Submit your article to this journal



Article views: 724



View related articles



View Crossmark data



Citing articles: 7 View citing articles

RNAi for the Treatment of People with Hemophilia: Current Evidence and Patient Selection

Sara Boyce¹, Savita Rangarajan²

¹Haemophilia Comprehensive Care Centre, University Hospital Southampton, Southampton, UK; ²Faculty of Medicine, University of Southampton, Southampton, UK

Correspondence: Sara Boyce, Email sara.boyce@uhs.nhs.uk

Abstract: Severe hemophilia is associated with spontaneous, prolonged and recurrent bleeding. Inadequate prevention and treatment of bleeding can lead to serious morbidity and mortality. Due to the limitations of intravenous clotting factor replacement, including the risk of inhibitory antibodies, innovative novel therapies have been developed that have dramatically changed the landscape of hemophilia therapy. Ribonucleic acid interference (RNAi) has brought the opportunity for multiple strategies to manipulate the hemostatic system and ameliorate the bleeding phenotype in severe bleeding disorders. Fitusiran is a RNAi therapeutic that inhibits the expression of the natural anticoagulant serpin antithrombin. Reduction in antithrombin is known to cause thrombosis if coagulation parameters are otherwise normal and can rebalance hemostasis in severe hemophilia. Reports from late stage clinical trials of fitusiran in hemophilia A and B participants, with and without inhibitory antibodies to exogenous clotting factor, have demonstrated efficacy in preventing bleeding events showing promise for a future “universal” prophylactic treatment of individuals with moderate-severe hemophilia.

Keywords: hemophilia, RNAi, siRNA, fitusiran, inhibitor, ribonucleic acid interference, small integral RNA

Introduction

Hemophilia A and B are inherited X-linked recessive bleeding disorders caused by mutations in coagulation factor VIII (hemophilia A) and IX (hemophilia B). These are essential proteins of the blood coagulation system that involves a cascade of zymogen activation that leads to the production of the serine protease thrombin, and ultimately cross-linked fibrin to form a stable blood clot.¹ Functional deficiency in coagulation factors VIII and IX results in abnormal hemostasis and excessive bleeding. In untreated severe hemophilia, where factor levels are <1% of normal, spontaneous bleeding occurs that frequently affects joints and can involve critical organs. A similar bleeding phenotype, with less frequent spontaneous bleeding, can occur in moderate hemophilia where factor levels are 2–5%. Onset of bleeding often begins in infancy, and from early childhood recurrent spontaneous joint bleeding can develop with consequential chronic painful and disabling joint arthropathy.² The current standard of treatment is replacement of the deficient factor VIII or IX with recombinant or plasma-derived factors. Clotting factor replacement is effective at preventing and treating the complications of hemophilia,³ but usually requires regular intravenous infusions 2–4 times a week with standard half-life factors. Extended half-life factors reduce the frequency of infusions, but individuals with severe hemophilia A still require intravenous prophylaxis 2–3 times a week.^{4,5} Frequent intravenous treatment is inconvenient and even distressing, particularly for infants, young children and their parents/caregivers where central venous access is often required that carries risk of infection.⁶ Lack of compliance comes hand-in-hand with difficulties of administering treatment and the complications of bleeding ensue.⁷ Additionally there is a risk of alloantibodies against the factor treatment, termed inhibitors, that occurs in approximately 30% of people with severe hemophilia A and 10% in severe hemophilia B.^{8,9} Inhibitors render standard treatment ineffective making patients more prone to severe and disabling bleeding, and treatment to eliminate inhibitors is costly,

invasive and has a high failure rate of approximately 20–40% in hemophilia A¹⁰ and 70% in hemophilia B.¹¹ Apart from immune tolerance therapy to eliminate inhibitors conventional treatment has been to administer bypassing agents, recombinant factor VIIa or activated prothrombin complex concentrate (aPCC) either on demand (OD) for bleeding, or as prophylaxis¹² that, again, is costly and invasive, requiring good venous access.

Due to the limitations of traditional treatments for hemophilia A and B these disorders have become a target for innovative strategies to overcome these challenges. The explosion of novel treatments that can transform the lives of people with hemophilia (PwH) has made this a compelling area of medicine over recent years. A major breakthrough in the management of haemophilia A and inhibitors arrived with the development of a bispecific antibody that partially mimics factor VIII activity, emicizumab (F.Hoffman-La Roche manufactured by Genentech).¹³ This treatment is the first licensed subcutaneous treatment for hemophilia and has become an established prophylactic treatment in inhibitor and non-inhibitor patients.^{14,15}

Making PwH free of regular prophylactic treatment altogether is now an attainable goal with gene therapy. As hemophilia is a monogenic disorder it has always been an attractive target for gene therapy and immense progress has been made in its clinical development.¹⁶ Though gene therapy has demonstrated great promise as a potential cure for hemophilia, immunological responses to the viral vector can lead to treatment failure and emerging long-term data has shown responses may not be sustained in hemophilia A.¹⁷ Additionally there are concerns regarding long-term safety of gene therapy, particularly viral integration into the host genome and oncogenesis.¹⁸ Gene therapy may be an opportunity to overcome the barriers to successful treatment of inhibitors and the efficacy of gene therapy in this patient population is being studied (ClinicalTrials.gov Identifier: NCT03734588).^{19,20}

Emicizumab has had great success due to the convenience of administration and stable pharmacological effect, which has fueled the demand for other treatments administered in a similar manner that can encompass a broader patient population. This can be achieved by targeting the natural inhibitors of coagulation. Normal hemostasis relies on an intricate balance of the generation of thrombin through the activation of coagulation proteins and their inhibition with regulatory proteins.²¹ Deficiency of the key regulatory anticoagulant proteins are known to increase the risk of venous thrombosis in a person with otherwise normal coagulation proteins.²² In hemophilia these deficiencies have been shown to ameliorate the hemophilia bleeding phenotype.²³ Manipulating natural inhibitors of coagulation has been demonstrated to effectively restore functional hemostasis using antibody neutralization of tissue factor pathway inhibitor²⁴ and protease nexin 1,²⁵ activated protein C specific serpin inhibition²⁶ and antithrombin inhibition by nanobodies.²⁷ Another strategy to rebalance hemostasis in hemophilia is to prevent the expression of the coagulation-inhibitory serpins is by utilizing ribonucleic acid interference (RNAi).

RNA Interference

RNAi, otherwise known as post-transcriptional gene silencing, is a highly conserved pathway whereby expression of a particular gene is inhibited through the targeting and destruction of an mRNA molecule by an RNA molecule.²⁸ This ancient process protects eukaryotic cells against invasion by exogenous genes, such as viral DNA or double-stranded RNA, and regulates expression of protein-coding genes.²⁹ Gene expression is inhibited when short RNA molecules, either micro RNAs (miRNA) or small integral RNAs (siRNA) bind to and functionally inactivate endogenous mRNA.

The concept of RNAi arose when, after attempts to overexpress the chalcone synthase gene in *Petunia* plants, expression of pigment was inhibited resulting in white flowers.³⁰ The term RNAi was coined by biotechnologist Craig Mello and Andrew Fire in 1998 with the discovery that double-stranded RNAs were the cause of post-translational gene silencing in the nematode worm *Caenorhabditis elegans*.³¹ This RNAi phenomenon led to the discovery of siRNA, typically double-stranded RNA molecules 20–25 nucleotides in length, that could induce RNAi silencing in mammalian cells.^{32,33} It was recognized siRNAs can potentially suppress any disease-related gene of interest and it has been a long and complex process to harness the widespread therapeutic possibilities. Unmodified siRNA is rapidly degraded and will not accumulate into the intended tissue.²⁹ In early clinical studies off-target effects were demonstrated with unmodified or slightly modified siRNA-targeting vascular endothelial growth factor A and R1 (VEGFA and VEGFR1) for local administration in age related macular degeneration.³⁴ Targeted siRNA against VEGFA or VEGFR1 to suppress choroidal neovascularization from age related macular degeneration via Toll-like receptor 3 and its adaptor molecule TRIF induced

secretion of proinflammatory cytokines IL-12 and IFN γ .^{34,35} Further barriers against efficacy and safety include enzymatic degradation by endogenous nucleases, rapid clearance, immune recognition, and egress from the bloodstream into the desired tissue.^{29,36} Some of these barriers have been overcome with chemical modification of siRNAs that can reduce off-target toxicity and enhance specificity and activity without compromising the siRNA activity. Phosphonate and ribose modification prevents siRNA degradation and are utilized in the Alnylam Pharmaceuticals Standard Template Chemistry universal modification pattern.³⁷ These modifications have made the theory of siRNA a clinically applicable reality and revolutionized therapeutics by circumventing the challenges of small molecules or antibodies having to recognize complex protein conformations. This technology was successfully applied with the first FDA approved (August 2018) siRNA, patisiran (ONPATRO) to deliver chemically modified anti-transthyretin siRNA to treat hereditary amyloidogenic transthyretin amyloidosis with polyneuropathy.³⁸ Patisiran employs lipids to protect entrapped siRNA from nuclease attack and renal clearance and transport to target tissue.²⁹ After intravenous administration of patisiran ionizable lipid nanoparticles associate with apolipoproteins facilitating endocytosis into liver.³⁹ During this period of siRNA development therapeutic applications in hemostasis had been explored. Lipid-based reagents had similarly allowed successful delivery of siRNAs to mouse livers in the first synthetic siRNA study on modifying the expression of liver coagulation proteins via hepatocyte nuclear factor 4a and CCAAT/enhancer binding protein 2 transcription factors.⁴⁰ Using the same mode of delivery siRNA silencing of the genes for the serpins protein C and antithrombin led to severe coagulopathy and macrothrombosis in multiple tissues in mice.⁴¹

Another method to surmount the evolutionary defenses to keep invading RNA outside of the cell was the design of N-acetylgalactosamine (GalNAc)-siRNA conjugates.⁴² Suppressing the expression of proteins produced by hepatocytes requires facilitating the entry of siRNA circulating in the blood stream after parenteral administration into the cytoplasm of hepatocytes; GalNAc was utilized for this purpose as it avidly binds to the Asialoglycoprotein, which is highly expressed on hepatocytes and endocytosed. GalNAc-siRNA conjugates can thus enter the hepatocyte and target cellular RNA and influence protein expression. This was successfully utilized in givosiran, the second FDA approved siRNA to treat acute hepatic porphyrias.⁴³

Fitusiran

Fitusiran (ALN-AT3, Alnylam/Sanofi) is a RNAi therapeutic developed for prophylaxis of bleeding in hemophilia A and B. This synthetic siRNA is covalently linked to a triantennary GalNAc ligand. The GalNAc-siRNA conjugate specifically targets antithrombin messenger RNA (mRNA) to lower antithrombin production (Figure 1). Antithrombin, encoded by the SERPINC1 gene, is a serpin produced by the liver that has an essential role in maintaining the balance of hemostasis. It functions as a natural anticoagulant serpin by potent inhibition of thrombin, and to other serine proteases including factors Xa and IXa. Antithrombin has a half-life of 2–3 days, normal plasma concentrations are 112–140 $\mu\text{g/mL}$ and the standard reference range is 80–120% of normal.⁴⁴ Individuals with inherited deficiency of antithrombin usually have levels of 40–60%; this carries a lifetime risk of venous thrombosis and is well known to cause a more severe thrombotic tendency than other inherited thrombophilias.^{45,46} Homozygous antithrombin deficiency usually causes in utero death, but rare cases have been reported.⁴⁴ Examination of the small number of reported cases of individuals with severe hemophilia and co-inherited thrombophilia has indicated reduction in the severity of their bleeding phenotype.^{23,47,48} Murine studies showed subcutaneous injection of fitusiran caused potent and durable reduction of antithrombin in both wild-type and hemophilia A mice, with an associated increase in thrombin generation and enhanced hemostasis.⁴⁹ In the Phase I dose escalation study to evaluate the safety and tolerability of subcutaneous fitusiran in healthy volunteers and non-inhibitor moderate-severe adult hemophilia A and B participants antithrombin levels were reduced by approximately 50% at doses between 0.015mg/kg and 1.8mg/kg (Table 1).⁵⁰ Peak fitusiran plasma levels occurred 2–6 hours after administration and rapidly decreased with a mean elimination half-life of 2.6–5.3 hours. Plasma levels of fitusiran increased and antithrombin decreased in a dose-dependent manner in the hemophilia patients (Table 1). Lowering of antithrombin was associated with increased thrombin generation at similar levels in hemophilia A and B patients. When antithrombin levels were reduced >75% from baseline peak thrombin generation values were at the lower end of the range observed in the healthy participants and thrombin generation values were similar to those described in mild hemophilia.⁵¹ There was a prolonged pharmacological effect with AT recovery at a median slope of 10–15% per month after fitusiran discontinuation indicating this treatment offers consistent hemostasis over time. Further exploratory analysis suggested this translates as fewer bleeding

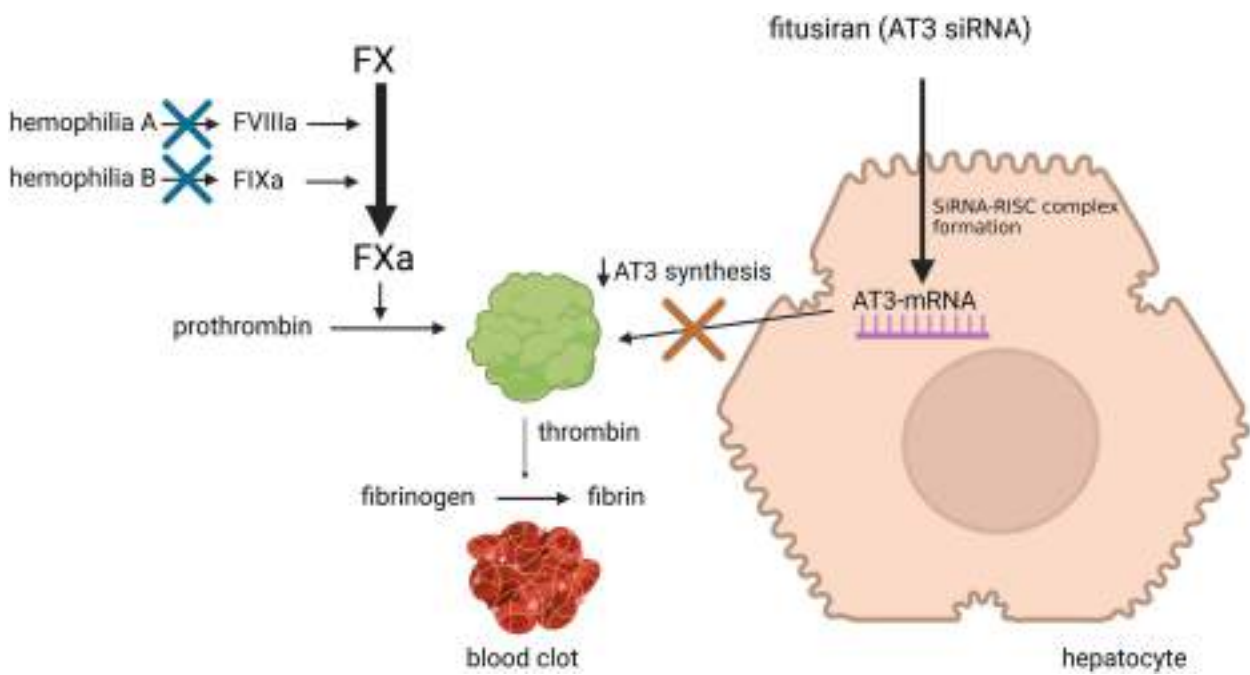


Figure 1 Diagram of the mechanism of action of fitusiran. After entering the hepatocyte the GaINAc- siRNA conjugate binds with a ribonucleoprotein forming a RNA-induced silencing complex (RISC). It targets AT3 mRNA and silences the gene, thus inhibiting antithrombin production. Consequently FIXa, Xa and thrombin lack inhibition and there is increased generation of thrombin to form blood clots. Created with BioRender.com.

episodes in patients, and reported episodes were successfully treated with clotting factor replacement. Fitusiran was well tolerated with injection site reactions and arthralgia being the most reported adverse events. Transient increases in liver aminotransferase was observed in 36% of participants and one participant had reactivation of hepatitis C infection that was thought unrelated to the study drug. No patients developed anti-fitusiran antibodies.

Due to the small sample size the phase I study was extended to evaluate the long-term safety and tolerability. In this extended study fatal cerebral venous sinus thrombosis occurred after factor VIII concentrate was repeatedly administered after an initial misdiagnosis of subarachnoid hemorrhage and all fitusiran trials were temporarily halted.⁵² The US Food and Drug Administration allowed the studies to continue after the trial protocol was amended to protect participants from the risk of thrombosis. For bleeding episodes lower doses of factor must be used; factor VIII 10–20 IU/kg or factor IX 20–30 IU/kg.⁵³ In vitro data on thrombin generation of bypassing agents and antithrombin reduction means it is likely lower doses are required⁵⁴ so aPCC 30–50 units/kg (standard dose 50–100 units/kg) or Factor VIIa < 45 mg/kg (standard

Table 1 Comparison of Median Nadir Lowering of at with Varying Fitusiran Dosing Regimens

Fitusiran Dose	Frequency	Mean Nadir Reduction AT	Median Peak Thrombin Height with > 75% AT Reduction
0.075mg/kg	Weekly	61%	67–68nM
0.225mg/kg	Monthly	70%	
1.8mg/kg	Monthly	89%	
50mg	Monthly	82%	
80mg	Monthly	87%	

Notes: There was a mean AT reduction from baseline of 87+ 1%⁴⁹ and 87.4%⁵⁴ in the two phase I studies. The median peak thrombin height range for healthy volunteers was 65–210nM; thrombin generation at > 75% reduction of AT from baseline by fitusiran was similar to lower healthy range.

dose 90mg/kg) should be given for bleeding in participants with inhibitors. Should a thrombotic episode occur in a PwH receiving fitusiran AT concentrate should be administered aiming for AT levels of 80–120%, in combination with anticoagulation and clotting factor or BPA replacement.

The trial program was extended to an open label Phase I, part D, study of efficacy in hemophilia participants with inhibitors.⁵⁵ Participants were treated with once monthly fixed subcutaneous doses of fitusiran at 50mg or 80mg. Again, the most common adverse events were mild and transient injection site reactions and increases in liver aminotransferases that were all assessed as mild to moderate reactions that did not require fitusiran suspension. The hepatotoxicity of GalNAc- siRNA has been shown as mainly due to RNAi-mediated off target gene silencing.⁵⁶ Due to the proportion of PwH with a history of hepatitis C infection, with 12/17 participants affected, this is thought to contribute to the risk of transaminitis related to this liver-specific therapeutic. Elevated d-dimer was measured as a potential predictor of thrombosis and was observed in 47%, more with the 80mg dose (55%) compared to the 50mg dose (33%). As d-dimer is also an acute phase reactant the clinical significance of this is unknown. Peak plasma levels were similar to the phase I study at 4 hours. The mean nadir AT level was 18% in the 50mg dose group and 12.5% in the 80mg dose group corresponding as 82% and 87.4% reduction from baseline (Table 1). As demonstrated previously reduced AT correlated with increased thrombin generation. Overall bleeding episodes were reduced with an improvement in quality of life; 65% participants reported no bleeding episodes (Table 2). The median aPCC dose used was 28.6 U/kg and no thrombotic events occurred. This study suggested a fixed monthly fitusiran dose is safe and efficacious for long-term treatment of inhibitor and non -inhibitor hemophilia patients.

The ATLAS trial program continued to expand (Table 3) and recently the findings of the Phase III ATLAS-A/B (ALN-AT3SC-004) study of the safety and efficiency of fitusiran in hemophilia A and B participants aged >12 years without inhibitors and previously receiving standard OD factor treatment were presented.⁵⁷ Participants were randomized to receive their usual OD treatment or monthly fitusiran 80mg s/c. Of the 79 participants that received fitusiran 50.6% did not have any bleeding episodes requiring factor treatment compared with 5% in the OD arm. The median annualized bleeding rate (ABR) was 0.0, with a clinically meaningful and statistically significant 89.9% reduction in ABR compared to the OD arm. No thrombotic events occurred during the study.

The ATLAS-INH (ALN-AT3SC-003) study evaluated the safety and efficacy of fitusiran in inhibitor patients >12 years and randomized participants to receive either 80mg fitusiran monthly or OD bypassing treatment.⁵⁸ Again, there was a significantly statistical reduction in ABR of 90.8% and 65.8% and fitusiran-receiving participants had zero bleeds compared with 5.8% in the OD arm. Seven patients in the fitusiran arm had treatment emergent serious adverse effects, including subclavian vein thrombosis and thrombosis. One patient with a spinal vascular disorder and thrombosis had study treatment discontinuation.

Table 2 Dosing Regimens and Bleeding Events in Published Fitusiran Studies

Study		Dosing Regimen	Bleeding Events Reported by Participant	Median ABR
Pasi et al 2017 ⁵⁰	Phase I dose escalation	Part B Once weekly 0.015, 0.045 or 0.075mg/kg weekly		
		Part C Once monthly 0.225, 0.45, 0.9 or 1.8mg/kg or Fixed dose 80mg monthly	67% reported no spontaneous bleeds 56% no bleeding events	0
Pasi et al 2020 ⁵⁵	Phase I part D	Once monthly 50mg or 80mg	65% no bleeding events	0
Young et al 2021 ⁵⁸ (ATLAS-INH)	Phase 3 open label	Once monthly 80mg	65.8% zero treated bleeding events	0
Srivastava et al 2021 ⁵⁷ (ATLAS-A/B)	Phase 3 open label	Once monthly 80mg	50.6% no bleeds that required treatment	0

Table 3 A Summary of the ATLAS program Fitusiran Studies

Trial	Phase	Subjects	Age	Inhibitors	Status
ALN-AT3SC-001	1	3 healthy subjects 42 Hemophilia A/B	≥ 18 years	Non-inhibitor	Complete
ALN-AT3SC-002	2	Hemophilia A/B Completed and tolerated study drug dosing in ALN-AT3SC-001	≥ 18 years	Non-inhibitor	Ongoing
ALN-AT3SC-003	3	Hemophilia A/B On demand bypassing agents	≥ 12 years	Inhibitors	Ongoing
ALN-AT3SC-004	3	Hemophilia A/B On demand factor replacement	≥ 12 years	Non-inhibitor	Ongoing
ALN-AT3SC-009	3	Hemophilia A/B with previous factor or bypassing agent prophylaxis	≥ 12 years	Inhibitor and non- inhibitor	Ongoing
ALN-AT3SC 005	3	Hemophilia A/B Previous participation in ALN-AT3SC-003 or ALN-AT3SC-004 or ALN-AT3SC-009	≥ 12 years	Inhibitor and non- inhibitor	Ongoing
ATLAS-PEDS	2/3	Hemophilia A/B	1-12 years	Inhibitor	Ongoing

Notes: Three global Phase 3 studies are ongoing, including 1) ALN-AT3SC-003 (ClinicalTrials.gov: NCT03417102) adolescents and adults ≥ 12 years with hemophilia and factor VIII or IX inhibitors previously treated with on-demand bypassing agents (BPA) 2) ALN-AT3SC-004 (ClinicalTrials.gov: NCT03417245): adolescents and adults with hemophilia without inhibitory antibodies previously treated with on-demand factor replacement therapies; 3) ALN-AT3SC-009 (ClinicalTrials.gov identifier NCT03549871): a single-arm, one way crossover study for adolescents and adults with hemophilia with or without inhibitors, and 4) ATLAS-PEDS (ClinicalTrials.gov identifier : NCT03974113) children ages 1–12 years with hemophilia and inhibitors. Bypassing agents (BPA) are recombinant factor VIIa or factor eight inhibitor bypassing agent (FEIBA)

Fitusiran in Other Rare Inherited Bleeding Disorders

As fitusiran does not target a specific coagulation factor it should theoretically prevent bleeding in other rare bleeding disorders. Addition of fitusiran to the plasma of patients severely deficient in factor V, VII or X has demonstrated improvement in thrombin generation.⁵⁹ AT-targeting RNAi may therefore be a therapeutic option for patients with rare bleeding disorders requiring prophylaxis where there is limited or no availability of clotting factor concentrate.

Other Strategies for RNAi Therapeutics in Hemophilia

Protein S

Protein S (PS) is a natural anticoagulant serpin encoded by the *PROS1* gene. It regulates the generation of thrombin by acting as a cofactor for activated protein C in the inactivation of coagulation factors Va and factor VIIIa.⁶⁰ It is also a cofactor for tissue factor pathway inhibitor that inhibits factor Xa.⁶¹ Homozygous PS deficiency can cause purpura fulminans and fatal disseminated intravascular coagulation (DIC).⁶² Heterozygous PS deficiency is associated with venous thromboembolic events.²² Like AT this regulator of hemostasis is an attractive target for RNAi therapeutics in the prevention of hemophilia-associated bleeding. Murine PS siRNA administered to hemophilia A ($F8^{-/-}$) and hemophilia B ($F9^{-/-}$) mice reduced bleeding in tail-bleeding assays, prevented intraarticular bleeding and PS expression was reduced in both the plasma and synovium.⁶³ High expression of PS and TFPI by synovial cells was found indicating this PS as a RNAi therapeutic target may further protect the joints in individuals prone to hemophilic arthropathy. Further studies to evaluate the safety of GalNAc conjugated to PS siRNA showed it was well tolerated mice with no evidence of DIC making further progress towards Phase I trials.⁶⁴

Heparin Cofactor II

Heparin cofactor II (HCII) is a serpin secreted by the liver and encoded by the *SERPIND1* gene.⁶⁵ Like AT, HCII has an essential role in regulating hemostasis through inhibition of thrombin.⁶⁶ HCII inactivates thrombin less efficiently than AT but glycosaminoglycans, such as heparin and dermatan sulfate enhance its effect. Dermatan sulfate is found mainly in the walls of blood vessels and has a particularly potent catalytic effect catalyzing HCII. This indicates HCII primarily exerts its effect in connective tissue. Like the better described inherited thrombophilias, deficiency of HCII has been associated with clinical thrombosis,⁶⁷ but other studies have shown the frequency of HCII deficiency in people with thrombosis is similar to healthy individuals.⁶⁸ The effect of RNAi on HCII has been studied in mice by conjugating siRNA-HCII with GalNAc.⁶⁹ This study suggested that by inhibition of HCII thrombin generation could be upregulated and hemostatic ability improved thus making HCII a potential target for hemophilia treatment.

Selection of Hemophilia Patients for RNAi Therapy

The development of plasma fractionation in the 1970s made the quality of life and life expectancy of PwH begin to increase. Transmission of hepatitis B and C and HIV through contaminated blood products to treat hemophilia tragically caused the loss of many lives. Now with recombinant clotting factors and successful antiviral therapies a significant rise in life expectancy is being seen.⁷⁰ As the hemophilia population ages, with associated co-morbidities, there are added complexities to their care. The ATLAS trial participants do not represent the entire hemophilia population as they were carefully selected by their clinicians and trial criteria, with key exclusion of inherited thrombophilia and thrombotic history. Fitusiran, therefore, cannot be presumed to be a “one size fits all” prophylactic treatment for severe hemophilia. As Phase IV and real-world data are accumulated clinicians are likely to select patients in line with the ATLAS program enrollment criteria. As inherited thrombophilia affects over 7% of certain populations some PwH will not be able to receive fitusiran based on thrombophilia testing alone.^{71,72} Data is needed to examine if the least prothrombotic and more frequent factor V Leiden and prothrombin G20210A mutations in conjunction with siRNA antithrombin suppression translates as a clinical thrombotic risk. The risk of thrombosis demonstrated in the ATLAS trials can be minimized through screening patients for thrombotic risk factors, modification of cardiovascular risk factors and cautious treatment of bleeding episodes. As well as the need to have a normal baseline AT prior to commencing fitusiran the AT response should be monitored in clinical practice to select which patients can safely continue fitusiran. Evaluation of the

thrombotic events related to fitusiran suggest there is association with AT levels of <10%. Therefore, as with ongoing trials, patients with AT levels of <15% should have fitusiran withdrawn. It has been reported to achieve the desirable ABR while minimizing adverse thrombotic events there should be an upper AT threshold with target levels of 35.⁷³ Future studies aim to further mitigate the risk of thrombosis by starting with 50mg dosing 2 monthly, and adjusting the dose and frequency based on the AT levels. The outcome of this study will guide clinicians on whether lower initial doses and tight control of AT target levels will minimize thrombosis risk enough to encompass a broader population of hemophilia patients, particularly those with cardiovascular risk factors, including rising age.

The lifestyle of a PwH may impact their selection for treatment. Though it has been established the risk of spontaneous bleeding with fitusiran is low, in clinical practice we are so far unable to precisely quantify the risk of bleeding from activities with high-risk physical impact. PwH who frequently play sports that carry a significant risk of head injury may be advised to use clotting factors prior to activity so there is confidence of a normal level of hemostasis, e.g., factor VIII or IX levels measurable at >50%. The same may apply to a PwH with an occupation that puts them at risk of major injury and bleeding.

It also needs to be considered increased bleeding risk can emerge in the ageing population, such as hemorrhagic stroke, bowel angiodysplasia or malignancy.⁷⁴ These co-morbidities alongside hemophilia may require the need for prophylaxis for the first time in the life of a PwH, or frequency of prophylaxis may need to be increased. Venous access is often challenging in the elderly, and vein fragility along with restricted joint movements due to osteoarthritis or hemophilic arthropathy may render self-treatment with intravenous infusions unfeasible and makes subcutaneous fitusiran, every 1–2 months, as attractive and convenient option. In these cases the bleeding and thrombotic risks would need to be carefully weighed against each other before a treatment decision is made.

Difficulties with venous access is not restricted to the elderly and is a major challenge in the early years of life. Traditional treatment of inhibitors, that most commonly affects children, requires high frequency intravenous infusions of clotting factor and/or BPAs. Recent data has reported the frequency of inhibitors in hemophilia B to be higher than previous findings;⁹ emicizumab cannot be used in this population and it is therefore likely they will be prioritized for fitusiran due to the unmet need and the high potential to turnaround the quality of life of the PwH and their caregivers.

PwH in the ATLAS trial programme were under the care of hemophilia centres and able to receive high quality medical care. This does not reflect global hemophilia care and access is a worldwide problem. Only 30% have access to clotting factor replacement therapy⁷⁵ and up to 10% of children with severe hemophilia in the developing world sustain intracranial hemorrhage.⁷⁶ Fitusiran could benefit PwH in developing countries with limited availability of medical care as the infrequent dosing and ease of administration has the potential to mitigate some of the difficulties with refrigerated storage and access to hospitals for instance with mobile monthly dosing clinics.

Conclusion

The goal of hemophilia therapy is to produce steady state hemostasis through the restoration of thrombin. With unprecedented progress in hemophilia treatments there has been a paradigm shift with expectations rising to aim for comparable quality of life to people without hemophilia.¹² RNAi therapeutics have been a major contributor to this change in landscape with numerous possibilities of rebalancing hemostasis through targeted suppression of serpin mRNA. This not only provides opportunities in the improving the care of PwH, but other people with rare severe bleeding disorders that have limited treatment options. Fitusiran is in late stages of clinical development and has been studied in over 200 participants in the international ATLAS clinical program. It has demonstrable efficacy in preventing bleeds through targeted reduction in antithrombin and restoration of thrombin generation in adolescents and adults with severe-moderate hemophilia A and B. Fitusiran differs from hemophilia treatments licensed to date as it is not only effective if inhibitors are present but also in both hemophilia A and B. If fitusiran is shown to be effective and safe in children, as is currently being assessed in Phase II and III studies, it has the potential to be an almost universal prophylactic treatment for hemophilia, with some caveats in patient selection. As the effective dosing regimens of 50mg and 80mg given subcutaneously every 1–2 months are much less frequent than standard prophylaxis with clotting factor infusions it could transform the quality of life of PwH, particularly for those with inhibitors. Long-term

morbidity from hemophilic arthropathy may be prevented with the greatest benefit offered to those with severe hemophilia managed with OD clotting factor treatment. Infrequent injections with sustained pharmacological effects has the additional benefit of granting ‘hemophilia-free days’, where the PwH is relieved of the psychological burden of their disease.⁷⁷

Overall fitusiran may transform lives of PwH across the world but should be prescribed after careful consideration, with particular attention to thrombotic risk factors, and with ongoing caution until there is enough data to enable clinicians to confidently select patients appropriately.

Disclosure

Dr Sara Boyce reports grants from Sangamo Therapeutics Ltd, personal fees, advisory board and speaker fees from CSL Behring, outside the submitted work. Dr Savita Rangarajan reports being a principal investigator in ongoing clinical Trials from Sanofi, during the conduct of the study; consultant from Reliance Life Sciences, advisory board from Pfizer, Sigilon, and Takeda, outside the submitted work. The authors report no other conflicts of interest in this work.

References

- Hoffman M, Monroe DM. Coagulation 2006: a Modern View of Hemostasis. *Hematol Oncol Clin North Am.* 2007;21(1):1–11. doi:10.1016/j.hoc.2006.11.004
- Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus Episodic Treatment to Prevent Joint Disease in Boys with Severe Hemophilia. *N Engl J Med.* 2007;357(6):535–544. doi:10.1056/NEJMoa067659
- Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. *J Thromb Haemost.* 2009;7(3):413–420. doi:10.1111/j.1538-7836.2008.03270.x
- Young G, Mahlangu JN. Extended half-life clotting factor concentrates: results from published clinical trials. *Haemophilia.* 2016;22:25–30. doi:10.1111/hae.13028
- Collins P, Chalmers E, Chowdary P, et al. The use of enhanced half-life coagulation factor concentrates in routine clinical practice: guidance from UKHCDO. *Haemophilia.* 2016;22(4):487–498. doi:10.1111/hae.13013
- Khair K, Ranta S, Thomas A, Lindvall K; PedNet study group. The impact of clinical practice on the outcome of central venous access devices in children with haemophilia. *Haemophilia.* 2017;23(4):e276–e281. doi:10.1111/hae.13241
- García-Dasí M, Aznar JA, Jiménez-Yuste V, et al. Adherence to prophylaxis and quality of life in children and adolescents with severe haemophilia A. *Haemophilia.* 2015;21(4):458–464. doi:10.1111/hae.12618
- Osooli M, Berntorp E. Inhibitors in haemophilia: what have we learned from registries? A systematic review. *J Intern Med.* 2015;277(1):1–15. doi:10.1111/joim.12301
- Male C, Andersson NG, Rafowicz A, et al. Inhibitor incidence in an unselected cohort of previously untreated patients with severe haemophilia B: a PedNet study. *Haematologica.* 2020;106(1):123–129. doi:10.3324/haematol.2019.239160
- Peyvandi F, Makris M. Inhibitor development in haemophilia. *Haemophilia.* 2017;23:3. doi:10.1111/hae.13145
- Meeks SL, Batsuli G. Hemophilia and inhibitors: current treatment options and potential new therapeutic approaches. *Hematology.* 2016;2016(1):657–662. doi:10.1182/asheducation-2016.1.657
- Srivastava A, Santagostino E, Dougall A, et al. WFH Guidelines for the Management of Hemophilia. *Haemophilia.* 2020;26(S6):1–158. doi:10.1111/hae.14046
- Young G, Liesner R, Chang T, et al. A multicenter, open-label Phase 3 study of emicizumab prophylaxis in children with hemophilia A with inhibitors. *Blood.* 2019;134(24):2127–2138. doi:10.1182/blood.2019001869
- Callaghan MU, Negrier C, Paz-Priel I, et al. Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies. *Blood.* 2021;137(16):2231–2242. doi:10.1182/blood.202009217
- Holstein K, Albisetti M, Bidlingmaier C, et al. Practical Guidance of the GTH Haemophilia Board on the Use of Emicizumab in Patients with Haemophilia A. *Hämostaseologie.* 2020;40(05):561–571. doi:10.1055/a-1127-6476
- Leebeek FWG, Miesbach W. Gene therapy for hemophilia: a review on clinical benefit, limitations, and remaining issues. *Blood.* 2021;138(11):923–931. doi:10.1182/blood.2019003777
- Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. *N Engl J Med.* 2020;382(1):29–40. doi:10.1056/NEJMoa1908490
- Donsante A, Miller DG, Li Y, et al. AAV Vector Integration Sites in Mouse Hepatocellular Carcinoma. *Science.* 2007;317(5837):477. doi:10.1126/science.1142658
- Arruda VR, Samelson-Jones BJ. Gene therapy for immune tolerance induction in hemophilia with inhibitors. *J Thromb Haemost JTH.* 2016;14(6):1121–1134. doi:10.1111/jth.13331
- Batty P, Lillicrap D. Advances and challenges for hemophilia gene therapy. *Hum Mol Genet.* 2019;28(R1):R95–R101. doi:10.1093/hmg/ddz157
- Monroe DM, Hoffman M. What Does It Take to Make the Perfect Clot? *Arterioscler Thromb Vasc Biol.* 2006;26(1):41–48. doi:10.1161/01.ATV.0000193624.28251.83
- Khan S, Dickerman JD. Hereditary thrombophilia. *Thromb J.* 2006;4(1):15. doi:10.1186/1477-9560-4-15
- Shetty S, Vora S, Kulkarni B, et al. Contribution of natural anticoagulant and fibrinolytic factors in modulating the clinical severity of haemophilia patients. *Br J Haematol.* 2007;138(4):541–544. doi:10.1111/j.1365-2141.2007.06693.x
- Chowdary P. Inhibition of Tissue Factor Pathway Inhibitor (TFPI) as a Treatment for Haemophilia: rationale with Focus on Concizumab. *Drugs.* 2018;78(9):881–890. doi:10.1007/s40265-018-0922-6

25. Aymonier K, Kaweck C, Venisse L, et al. Targeting protease nexin-1, a natural anticoagulant serpin, to control bleeding and improve hemostasis in hemophilia. *Blood*. 2019;134(19):1632–1644. doi:10.1182/blood.2019000281
26. Polderdijk SGI, Adams TE, Ivanciu L, Camire RM, Baglin TP, Huntington JA. Design and characterization of an APC-specific serpin for the treatment of hemophilia. *Blood*. 2017;129(1):105–113. doi:10.1182/blood-2016-05-718635
27. Barbon E, Ayme G, Mohamadi A, et al. Single-domain antibodies targeting antithrombin reduce bleeding in hemophilic mice with or without inhibitors. *EMBO Mol Med*. 2020;12:4. doi:10.15252/emmm.201911298
28. Setten RL, Rossi JJ, Han S. The current state and future directions of RNAi-based therapeutics. *Nat Rev Drug Discov*. 2019;18(6):421–446. doi:10.1038/s41573-019-0017-4
29. Hu B, Zhong L, Weng Y, et al. Therapeutic siRNA: state of the art. *Signal Transduct Target Ther*. 2020;5(1):101. doi:10.1038/s41392-020-0207-x
30. Napoli C, Lemieux C, Jorgensen R. Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans. *Plant Cell*. 1990;2:279–289. doi:10.1105/tpc.2.4.279
31. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998;391(6669):806–811. doi:10.1038/35888
32. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*. 2001;411(6836):494–498. doi:10.1038/35078107
33. Caplen NJ, Parrish S, Imani F, Fire A, Morgan RA. Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc Natl Acad Sci*. 2001;98(17):9742–9747. doi:10.1073/pnas.171251798
34. Kleinman ME, Yamada K, Takeda A, et al. Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature*. 2008;452(7187):591–597. doi:10.1038/nature06765
35. Barakat MR, Kaiser P. VEGF inhibitors for the treatment of neovascular age-related macular degeneration. *Expert Opin Investig Drugs*. 2009;18(5):637–646. doi:10.1517/13543780902855316
36. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov*. 2009;8(2):129–138. doi:10.1038/nrd2742
37. Maraganore J. Reflections on Alnylam. *Nat Biotechnol*. 2022;40(5):641–650. doi:10.1038/s41587-022-01304-3
38. Weng Y, Xiao H, Zhang J, Liang XJ, Huang Y. RNAi therapeutic and its innovative biotechnological evolution. *Biotechnol Adv*. 2019;37(5):801–825. doi:10.1016/j.biotechadv.2019.04.012
39. Wittup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. *Nat Rev Genet*. 2015;16(9):543–552. doi:10.1038/nrg3978
40. Safdar H, Cheung KL, Vos HL, et al. Modulation of Mouse Coagulation Gene Transcription following Acute In Vivo Delivery of Synthetic Small Interfering RNAs Targeting HNF4a and C/EBPα. *PLoS One*. 2012;7(6):e38104. doi:10.1371/journal.pone.0038104
41. Safdar H, Cheung KL, Salvatori D, et al. Acute and severe coagulopathy in adult mice following silencing of hepatic antithrombin and protein C production. *Blood*. 2013;121(21):4413–4416. doi:10.1182/blood-2012-11-465674
42. Springer AD, Dowdy SF. GalNAc-siRNA Conjugates: leading the Way for Delivery of RNAi Therapeutics. *Nucleic Acid Ther*. 2018;28(3):109–118. doi:10.1089/nat.2018.0736
43. Sardh E, Harper P, Balwani M, et al. Phase 1 Trial of an RNA Interference Therapy for Acute Intermittent Porphyria. *N Engl J Med*. 2019;380(6):549–558. doi:10.1056/NEJMoa1807838
44. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review: AT DEFICIENCY. *Haemophilia*. 2008;14(6):1229–1239. doi:10.1111/j.1365-2516.2008.01830.x
45. Lijfering WM, Brouwer JLP, Veeger NJGM, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on absolute thrombotic risk for currently known thrombophilic defects in 2479 relatives. *Blood*. 2009;113(21):5314–5322. doi:10.1182/blood-2008-10-184879
46. Croles F, Borjas-Howard J, Nasserinejad K, Leebeek F, Meijer K. Risk of Venous Thrombosis in Antithrombin Deficiency: a Systematic Review and Bayesian Meta-analysis. *Semin Thromb Hemost*. 2018;44(04):315–326. doi:10.1055/s-0038-1625983
47. Escuriola Ettingshausen C, Halimeh S, Kurnik K, et al. Symptomatic onset of severe hemophilia A in childhood is dependent on the presence of prothrombotic risk factors. *Thromb Haemost*. 2001;85(2):218–220. doi:10.1055/s-0037-1615679
48. Franchini M, Montagnana M, Targher G, et al. Interpatient Phenotypic Inconsistency in Severe Congenital Hemophilia: a Systematic Review of the Role of Inherited Thrombophilia. *Semin Thromb Hemost*. 2009;35(03):307–312. doi:10.1055/s-0029-1222609
49. Sehgal A, Barros S, Ivanciu L, et al. An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia. *Nat Med*. 2015;21(5):492–497. doi:10.1038/nm.3847
50. Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy. *N Engl J Med*. 2017;377(9):819–828. doi:10.1056/NEJMoa1616569
51. Dargaud Y, Béguin S, Lienhart A, et al. Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. *Thromb Haemost*. 2005;93(03):475–480. doi:10.1160/TH04-10-0706
52. Alnylam Reports Patient Death in Fitusiran Clinical Study; 2017. Available from: <https://www.hemophilia.org/news/>. Accessed April 1, 2023.
53. Machin N, Ragni MV. An investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia A and B. *J Blood Med*. 2018;9:135–140. doi:10.2147/JBM.S159297
54. Livnat T, Sehgal A, Qian K, et al. Thrombin generation in plasma of patients with haemophilia A and B with inhibitors: effects of bypassing agents and antithrombin reduction. *Blood Cells Mol Dis*. 2020;82:102416. doi:10.1016/j.bcmd.2020.102416
55. Pasi KJ, Lissitchkov T, Mamonov V, et al. Targeting of antithrombin in hemophilia A or B with investigational siRNA therapeutic fitusiran—Results of the phase 1 inhibitor cohort. *J Thromb Haemost*. 2021;19(6):1436–1446. doi:10.1111/jth.15270
56. Janas MM, Schlegel MK, Harbison CE, et al. Selection of GalNAc-conjugated siRNAs with limited off-target-driven rat hepatotoxicity. *Nat Commun*. 2018;9(1):723. doi:10.1038/s41467-018-02989-4
57. Srivastava A, Rangarajan S, Kavakli K, et al. Fitusiran, an Investigational siRNA Therapeutic Targeting Antithrombin for the Treatment of Hemophilia: first Results from a Phase 3 Study to Evaluate Efficacy and Safety in People with Hemophilia a or B without Inhibitors (ATLAS-A/B). *Blood*. 2021;138(Supplement2):LBA–3. doi:10.1182/blood-2021-155018
58. Young G, Srivastava A, Kavakli K, Ross C, Sathar J, Tran H. Efficacy and safety of fitusiran prophylaxis, an siRNA therapeutic, in a multicentre phase 3 study (ATLAS-INH) in people with haemophilia A or B, with inhibitors. *ASH Plenary Sci Sess*. 2021:543.

59. Sridharan G, Liu J, Qian K, Goel V, Huang S, Akinc A. In silico modelling of the impact of antithrombin lowering on thrombin generation in rare bleeding disorders. *Blood*. 2017;130(Supplement 1):3659.
60. Ten Kate MK, Van Der Meer J. Protein S deficiency: a clinical perspective. *Haemophilia*. 2008;080512004759292. doi:10.1111/j.1365-2516.2008.01775.x
61. Hackeng TM, Sere KM, Tans G, Rosing J. Protein S stimulates inhibition of the tissue factor pathway by tissue factor pathway inhibitor. *Proc Natl Acad Sci*. 2006;103(9):3106–3111. doi:10.1073/pnas.0504240103
62. Mahasandana C, Veerakul G, Tanphaichitr VS, Suvatte V, Opartkiattikul N, Hathaway WE. Homozygous protein S deficiency: 7-year follow-up. *Thromb Haemost*. 1996;76(6):1122. doi:10.1055/s-0038-1650718
63. Prince R, Bologna L, Manetti M, et al. Targeting anticoagulant protein S to improve hemostasis in hemophilia. *Blood*. 2018;131(12):1360–1371. doi:10.1182/blood-2017-09-800326
64. Prince R, Schaeper U, Dames S, et al. Targeting Protein S Using Small Interfering RNA Is Well Tolerated and Protects Mice with Hemophilia a from Acute Hemarthrosis. *ASH Oral Abstr*. 2020:54.
65. Tollefsen DM. Heparin Cofactor II Modulates the Response to Vascular Injury. *Arterioscler Thromb Vasc Biol*. 2007;27(3):454–460. doi:10.1161/01.ATV.0000256471.22437.88
66. Rau J, Mitchell J, Fortenberry Y, Church F. Heparin Cofactor II: discovery, Properties, and Role in Controlling Vascular Homeostasis. *Semin Thromb Hemost*. 2011;37(04):339–348. doi:10.1055/s-0031-1276582
67. Lopaciuk S, Bykowska K, Kopeć M. Prevalence of heparin cofactor II deficiency in patients with a history of venous thrombosis. *Pol J Pharmacol*. 1996;48(1):109–111.
68. Tollefsen DM. Heparin Cofactor II Deficiency. *Arch Pathol Lab Med*. 2002;126(11):1394–1400. doi:10.5858/2002-126-1394-HCID
69. Lin W, Zhu R, Zhang Z, et al. RNAi targeting heparin cofactor II promotes hemostasis in hemophilia A. *Mol Ther Nucleic Acids*. 2021;24:658–668. doi:10.1016/j.omtn.2021.03.022
70. Kempton CL, Makris M, Holme PA. Management of comorbidities in haemophilia. *Haemophilia*. 2021;27(S3):37–45. doi:10.1111/hae.14013
71. Ridker PM. Ethnic Distribution of Factor V Leiden in 4047 Men and Women: implications for Venous Thromboembolism Screening. *JAMA*. 1997;277(16):1305. doi:10.1001/jama.1997.03540400055031
72. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *The Lancet*. 1995;346(8983):1133–1134. doi:10.1016/S0140-6736(95)
73. Pipe S, Srivastava A, Klamroth R, et al. Fitusiran, an Investigational siRNA Therapeutic Targeting Antithrombin: analysis of Antithrombin Levels and Thrombin Generation from a Phase 3 Study in People with Hemophilia A or B Without inhibitors [abstract]. *ISTH Congr*. 2022.
74. Philipp C. The Aging Patient with Hemophilia: complications, Comorbidities, and Management Issues. *Hematology*. 2010;2010(1):191–196. doi:10.1182/asheducation-2010.1.191
75. Pierce GF, Haffar A, Ampartzidis G, et al. First-year results of an expanded humanitarian aid programme for haemophilia in resource-constrained countries. *Haemophilia*. 2018;24(2):229–235. doi:10.1111/hae.13409
76. Andersson NG, Auerswald G, Barnes C, et al. Intracranial haemorrhage in children and adolescents with severe haemophilia A or B - The impact of prophylactic treatment. *Br J Haematol*. 2017;179(2):298–307. doi:10.1111/bjh.14844
77. Krumb E, Hermans C. Living with a “hemophilia-free mind” – the new ambition of hemophilia care? *Res Pract Thromb Haemost*. 2021;5(5):e12567. doi:10.1002/rth2.12567

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



“Severe Anemia: A Case Report of an Uncommon Precipitant of Schizophrenia Relapse”

Krittisak Anuroj, Siwat Chongbanyatcharoen & Romteera Chiencharoenthanakij

To cite this article: Krittisak Anuroj, Siwat Chongbanyatcharoen & Romteera Chiencharoenthanakij (2023) “Severe Anemia: A Case Report of an Uncommon Precipitant of Schizophrenia Relapse”, Journal of Blood Medicine, , 329-336, DOI: [10.2147/JBM.S407722](https://doi.org/10.2147/JBM.S407722)

To link to this article: <https://doi.org/10.2147/JBM.S407722>



© 2023 Anuroj et al.



Published online: 22 Apr 2023.



Submit your article to this journal



Article views: 566



View related articles



View Crossmark data



Citing articles: 1 View citing articles

“Severe Anemia: A Case Report of an Uncommon Precipitant of Schizophrenia Relapse”

Krittisak Anuroj , Siwat Chongbanyatcharoen, Romteera Chiencharoenthanakij

Department of Psychiatry, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok, Thailand

Correspondence: Krittisak Anuroj, Department of Psychiatry, Faculty of Medicine, Srinakharinwirot University, 62 Moo 7 Ongkharak Subdistrict, Ongkharak District, Nakhon Nayok, 26120, Thailand, Tel +6637385085 Ext. 60804, Email thymelancille@gmail.com

Abstract: A 48-year-old patient with stable residual schizophrenia experienced a syndromic psychosis relapse following an episode of severe combined immunohemolytic and pure red cell aplastic anemia, with a hemoglobin level of 4.7 g/dl. The anemia was attributed to her anti-HIV medication zidovudine. Her HIV infection had been well-controlled; no other organic precipitant of the psychosis was found. Following transfusion of 2 units of leukocyte-poor packed red cells, schizophrenia symptoms promptly recovered to her baseline. This was maintained at 3- and 6-month follow-ups without any need for antipsychotic dose adjustment. Following zidovudine discontinuation and a short course of oral prednisolone, her anemia gradually recovered.

Keywords: neuropsychiatric, psychiatric, hematologic, zidovudine, HIV infection

Plain Language Summary

The authors describe a case of stable schizophrenia that had a relapse due to severe anemia. Blood transfusion resulted in quick and complete recovery to the patient's baseline.

Introduction

A recent estimate projected that anemia affects 26.8% of the population worldwide and is associated with 58.6 million years lived with disability, making it a public health concern. The signs and symptoms of anemia vary by severity, onset and chronicity, and etiology of the condition. End-organ symptoms from poor perfusion can be present in those with chronic or progressive anemia, causing symptoms such as fatigue, decrease in stamina, light-headedness, dyspnea, exercise intolerance, or syncope.¹ Affecting the brain, anemia can manifest with neuropsychiatric presentations including mood disorders, anxiety disorders, and psychoses.²⁻⁶ Vitamin B12 deficiency, a cause of megaloblastic anemia, is known to cause psychosis regardless of the presence of anemia,⁷⁻⁹ through mechanisms related to one-carbon metabolisms and syntheses of neurotransmitters.^{10,11} Iron deficiency, another common entity, can affect dopamine receptor levels and disrupt functions of sensitive brain regions resulting in psychosis, again regardless of presence of anemia.^{12,13}

Schizophrenia is a debilitating psychiatric disease with core symptoms of delusion and hallucination, affecting approximately 1% of the world's population.¹⁴ At its onset, schizophrenia itself has been associated with low serum iron level, but not with anemia.^{12,15} However, data on association between schizophrenia relapse and anemia are scarce. A PubMed search performed on 23 October 2022 with search string “(anemia OR anemic) AND (schizophrenia OR schizophrenic OR schizophreniform)” yielded 194 results, among which only one article was relevant: a case report of a known schizophrenia patient with multiple comorbidities presenting with hallucination from sickle cell crisis in 1991. The case passed away during the admission, hence there were no data on treatment effectiveness or follow-ups.¹⁶ The current report complements the hematology literature on anemia and its role in triggering schizophrenia relapse.

The Case

The patient is a 48-year-old female who was diagnosed with schizophrenia in 2014. Initially, multiple antipsychotics were consolidated due to her severe symptoms, and the regimen has been in place ever since due to the presence of residual symptoms (Table 1). Her residual symptoms have been stable and included auditory hallucinations with intact insight and reality testing. Since the first episode, she has displayed good treatment compliance and never experienced

Table 1 Timeline of Patient's HIV Infection and Schizophrenia

Time	Medical Conditions	Schizophrenia
Late 2012	Diagnosed with HIV infection. Received lamivudine, stavudine, and efavirenz. CD4+ 72 cells/ul. Prophylactic fluconazole and double strength co-trimoxazole given.	N/A
2013	Asymptomatic, no opportunistic infection. Decreased co-trimoxazole to single strength.	Infrequently had hallucinations and irritability associated with alcohol use. Has yet to receive formal diagnosis or treatment.
2014	Asymptomatic, no opportunistic infection. CD4+ 377 cells/ul. VL 85 copies/mL. Discontinued fluconazole and co-trimoxazole.	Displayed severe delusion and hallucination. Hospitalized. Schizophrenia diagnosed. Treated with 6 mg/day of risperidone, 100 mg/month of haloperidol decanoate, and 50 mg/month of fluphenazine. Became alcohol-abstinent ever since.
2015	Asymptomatic, no opportunistic infection. CD4+ between 575 cells/ul. VL 56 copies/mL.	Residual auditory hallucinations with intact insight. No delusion or disorganized behavior. Functioned stably in housekeeping. Antipsychotic drug regimen unchanged. Good compliance
2016–2019	Asymptomatic, no opportunistic infection. CD4+ 880–1082 cells/ul. VL <40 copies/mL.	Stable
2020	Anti-HIV drugs were changed to lamivudine, zidovudine, and efavirenz due to supply issues. Asymptomatic, no opportunistic infection. CD4+ 1935 cells/ul. VL <20 copies/mL.	Stable
2021 – January 2022	Asymptomatic, no opportunistic infection. CD4+ 1270–1317 cells/ul. VL <20 copies/mL.	Stable
April 2022	Unremarkable. Reportedly compliant to treatment.	Increased irritability. Loss of insight into own condition. Responded to hallucination. Slow thinking and speech. Decreased housekeeping ability. Reported headache, fatigue, and cold intolerance. Reportedly compliant to treatment.
May 2022	Unremarkable. Reportedly compliant to treatment.	Increased headache and fatigue. Visited ER. Dismissed as schizophrenia relapse. No investigation carried out.
June 2022	Combined immunohemolytic and pure-red cell aplastic anemia were found; Hb 4.2 g/dl. Hospitalized. Received 2 units of LPRC; Hb raised to 7.8 g/dl. Zidovudine was implicated and discontinued. Anti-HIV regimen changed to tenofovir, emtricitabine, and efavirenz. Started on 60 mg/day of prednisolone for immunohemolysis.	Visited psychiatric OPD which led to investigations. Psychotic symptoms promptly improved after transfusion. No psychiatric medication changes. Discharged after 2 days.
July 2022	HIV: asymptomatic. No anemic symptoms. Hb was 7.3 g/dl with no signs of ongoing hemolysis. Prednisolone continued at same dose.	Stable (same as 2015–2021 baseline)

(Continued)

Table 1 (Continued).

Time	Medical Conditions	Schizophrenia
August 2022	HIV: asymptomatic. No anemic symptoms. Hb rose to 10.6 g/dl with no signs of ongoing hemolysis. Prednisolone discontinued.	Stable
September 2022	HIV: asymptomatic. No anemic symptoms.	Stable
December 2022	HIV: asymptomatic. No anemic symptoms. Hb rose to 12.0 g/dl with no signs of ongoing hemolysis. Normal reticulocyte counts.	Stable

Abbreviations: CD4+, CD4+ positive cell counts; VL, HIV viral loads; LPRC, leukocyte-poor packed red cell; Hb, hemoglobin level.

relapse. The clinical global impression-severity was 2 (borderline ill) and clinical global impression-improvement was 1 (marked improvement from initial encounter). The patient functioned stably in her housekeeping duties.

Her medical history was significant for HIV infection, which was diagnosed in 2012. Initial CD4+ count at the time of diagnosis was 72 cells/ul, which rose to 377 cells/ul in the first year of treatment with lamivudine, stavudine, and efavirenz. She never had any opportunistic infection. Past self-reported pill counts indicated excellent compliance. Antiretroviral regimen was changed once owing to changes in locally available formulary (Table 1) to lamivudine, zidovudine, and efavirenz in 2020. In the past 3 yearly laboratory investigations, her CD4+ cell counts were between 1163–1935 cells/ul and viral loads were all < 20 copies/mL. She had no other underlying diseases.

The current relapse began 2 months prior. Her daughter noted that the patient started to respond to (the ever present) hallucinations while becoming more irritable, yet displayed slower responses to stimuli; these symptoms did not show any signs of fluctuation, nor were disorientations noted. The patient reported that her hallucinations occurred more frequently, and that she lost the ability to differentiate between hallucinations and reality. She also complained of fatigue, headache, and cold intolerance. Noncompliance was ruled out as the daughter noted that the patient regularly took her medicines and went for monthly injections while remaining pill counts were consistent with follow-up visit date. The patient was brought to the emergency department twice for her somatic complaints. In both instances, two separate physicians focused more on the relapse of psychotic symptoms and only prescribed intramuscular haloperidol without performing laboratory investigation before discharging the patient. The patient later came for a regular psychiatric follow-up visit and reported the relapse and somatic symptoms. The psychiatrist ascertained that there was no compliance issue, nor was there comorbid use of tobacco, alcohol, or illicit substances that could have triggered a psychotic episode or affected pharmacokinetics of the prescribed medication. Examination was positive for systolic ejection murmur. There was no thyroid enlargement, peripheral edema, or abnormalities in lung auscultation. Neurological examination was normal. Investigation revealed a hemoglobin level of 4.7 g/dl with multiple microspherocytes seen in peripheral blood smear; there was no history of hereditary spherocytosis or notes of spherocytes in complete blood counts in the past 10 years. Direct Coomb's test was positive (3+). Reticulocyte count, however, was not elevated (reticulocyte 0.24%, absolute count 3000 cells/ul). Iron studies were normal. Vitamin B12 level was within intermediate range and was deemed unexplanatory of the low reticulocyte count. (The cause of borderline low vitamin B12 was attributed to her self-medication with omeprazole for dyspepsia). Combined autoimmune hemolytic anemia and pure red cell aplasia attributed to zidovudine was provisionally diagnosed. Infectious specialist ruled out possibilities of anemia and psychotic symptoms from HIV, as her infection had been under good control. As her homocysteine level, the marker of abnormality in one-carbon metabolism pathway and the implicated mechanism of psychosis from vitamin B12 deficiency, was within normal range, psychosis from the vitamin status was ruled out. No abnormalities were seen in serum electrolyte panels, thyroid functions, and liver functions, nor was there rhabdomyolysis as evaluated by serum creatine phosphokinase, lactate dehydrogenase and urine myoglobin, ruling out their contribution to psychosis and somatic symptoms.

Transfusion with 2 units of leukocyte-poor packed red blood cells was initiated. After her hemoglobin level rose to 7.8 g/dl over the course of the night the transfusion was carried out, her psychotic symptoms promptly abated: frequency

of hallucination became rare, insight and reality testing returned, and mood and affect became euthymic. The recovery was maintained throughout the admission. No adjustment in psychiatric medication was necessary. As zidovudine was implicated as etiology of anemia, the anti-HIV regimen was changed to tenofovir, emtricitabine, and efavirenz. 60 mg/d of prednisolone was given for immunohemolysis. At 2-week follow-up her hemoglobin level was similar to post-transfusion (7.3 g/dl) without any ongoing signs of hemolysis in blood smear, and schizophrenia symptoms were stable. At 3-months, and 6-months follow-ups, her schizophrenia remained in quiescence and her anemia gradually improved (Table 1). Self-reported compliance and leftover pill counts indicated good compliance.

Ethical Considerations

This case report was approved by Human Research Ethics Committee of Srinakharinwirot University (number: SWUEC-M-075/2565E). Written informed consent for publication of case details and any accompanying images was obtained from the patient during the period of stable psychiatric symptoms as per the ethics committee's approval. The authors' institution did not require approval to publish case detail.

Discussion

Zidovudine and Anemia

Anemia in the present case was attributed to zidovudine. The agent is known to cause anemia from multiple mechanisms, including immunohemolysis and bone marrow suppression. Latency from drug initiation can be years,^{17–21} which is consistent with presentation in our case. HIV infection itself has also been associated with development of immunohemolysis but only in severely immunocompromised hosts,^{22,23} which is unlikely as the patient's HIV infection had been under good control.

Immunohemolysis was evident from direct Coomb's test and typical peripheral blood smear. Pure red cell aplasia, another anemic condition known to be triggered by zidovudine, was inferred from the lack of reactive reticulocytosis to the 2-month course of anemia despite the absence of nutritional deficiency. The absence of bone marrow study results in this case, however, precluded a firm diagnosis of the latter condition.

Different Causes of Anemia and Psychosis

Reports on psychosis from anemia are still sparse. Most were related to megaloblastic anemia associated with vitamin B12 and folate deficiency ("megaloblastic madness").²⁴ Historic cases reported that psychoses in pernicious anemia were treatable with electroconvulsion.^{25,26} The response to the same treatment as schizophrenia spectrum psychoses may imply shared pathological processes. More recently, a report described a case with megaloblastic anemia presenting as subacute disorganized behaviors and memory impairment. The case improved after three days of vitamin B12 administration.²⁷ Normalization of hematopoiesis could not have occurred in such short period. This is also consistent with other reports in which psychoses occurred in isolated vitamin B12 deficiency without megaloblastic anemia,^{28–30} pointing to the deficiency's independent effect such as homocysteinemia due to problems in one-carbon metabolisms or abnormalities in synthesis of neurotransmitters.^{10,11,31} Similarly, the widely studied iron deficiency anemia is also implicated as the predisposing factor of schizophrenia,^{32–34} but the mechanism driving development of psychosis appears to be from iron status deviating brain developmental trajectories.^{13,35–37} Therefore, psychoses from these two entities likely followed mechanisms different from that of the present case.

There is a report of a case with exacerbated chronic familial hemolytic anemia (hemoglobin 3.7 g/dl) following *Clostridium perfringens* sepsis which caused psychosis. In contrast to our case, this patient developed paranoid psychosis, aggression, and disorientation after transfusion, and only improved with subsequent antibiotics treatment.³⁸ The clinical picture is likely that of delirium (as opposed to our case), which can be attributed to either anemia, sequelae of hemolysis, transfusion reaction, and/or sepsis.

A similar clinical situation was reported in a stable schizophrenia patient who developed combined megaloblastic and autoimmune hemolytic anemia. Despite having similarly low hemoglobin level (initially 6.5 g/dl, later dropped to 5.4 g/dl), this patient did not experience relapse of psychotic symptoms as reported in the present case despite having

hemoglobin level only slightly higher than our case.³⁹ The report stated that the patient's psychosis was in remission, a different baseline from our case whose residual symptoms had been present. The residual symptoms point to a more severe pathology that required greater compensatory functions and, possibly, a greater vulnerability to any loss.

Anemia, Cognitive Functions, and the Link with Psychological Insight

The mechanism by which anemia induced psychosis in the present case was possibly different from those in micro-nutrient deficiency or sepsis. The possibility that autoimmunity status mediated the exacerbation is also unlikely, as the psychosis resolved prior to initiation of prednisolone. We hypothesize the mechanism of impairment and recovery of cognitive function explaining the relapse and prompt recovery with transfusion. As previously noted, the patient's insight and reality testing only became impaired during this episode. In modern sense, the psychoanalytic-inclined term of reality testing and insight has now been viewed as an expression of cognitive functions.^{40–42} Recent views on schizophrenia have implicated the decline in cognitive functions as one of the triggers of conversion from high-risk states (including attenuated psychosis syndrome and brief limited intermittent psychotic symptoms) to schizophrenia.⁴³ If so, a decline in sensitive cognitive functions in a patient with established disease should also be able to trigger a schizophrenia relapse. In this case, the role of anemia was apparent. Anemia disrupts the oxygenation of the central nervous system, impairing cognitive functions. Two experiments examined reaction time and memory in iatrogenically-produced acute normovolemic anemia. These cognitive functions started to decline subtly at hemoglobin level of 6 g/dl (approximately 8 g/dl below mean pre-dilution concentration). Autotransfusion reversed the impairment, while administration of supplemental oxygen alone could also create partial improvement.^{44,45} This suggests that poor oxygenation contributed to the deficits. A further experiment found that the impaired cognitive functions were attributable to impaired central processing.⁴⁶ Although these studies have yet to explore the anemia's and transfusion's effects on other cognitive domains, it can be presumed that deficits and recoveries occur simultaneously. Unfortunately, these studies explored changes in cognition in short-term anemia. The present case's anemia had likely developed over the past two months and whether the experiment's findings are generalizable is not known. Chronic anemia can be followed by compensation and decompensation processes which can distort its effects on cognitive functions related to psychological insight. Nevertheless, data from commonly encountered chronic anemia such as iron deficiency, end-stage renal disease, sickle cell anemia, and thalassemia seem to indicate the presence of cognitive disturbances in these entities as well, although their disease-specific pathophysiology may also be operating on top of anemia itself.^{47–51} Chronic anemia in the elderly and oncologic patients has also been associated with reversible cognitive deficits.^{52,53}

The Present Case and Implications

From the psychiatric perspective, a disease relapse is a function of risk factors. For schizophrenia, common risk factors are medication discontinuation and substance use, which were not present in this case. Another strong factor is the disease's chronicity, which is assumed to be accompanied by ongoing pathological processes such as low-grade systemic inflammation and brain function deterioration.^{54–59} Moreover, the chronic use of antipsychotics and the patient's sedentary lifestyle could have led to metabolic derangements, potentially worsening the inflammatory condition.⁶⁰ Her HIV infection, although well controlled, can also exert subtle inflammatory disturbances that chronically deteriorate brain function and adds to the predisposition.⁶¹ These factors are then precipitated into a syndromic relapse by the emergence of severe anemia.

From her course of physical symptoms and relatively preserved level of consciousness, it was suspected that her anemia may have been gradually progressing over the past two months, concurrent with the re-emergence of psychotic symptoms. Due to the absence of symptom fluctuation and disorientation, the psychosis was unlikely a part of delirium. Therefore, the re-emergence of symptoms was diagnosed as schizophrenia relapse. Her reality testing, insight, and auditory hallucination recovered to baseline after transfusion raising hemoglobin level from 4.2 to 7.8 g/dl. The rapid improvement of insight could be a product of recovery of sensitive cognitive functions, similar to situations observed in iatrogenic normovolemic anemia experiments. Hallucination has been postulated as a disorder of brain regions generating inner speech, while the insight or the ability to tell hallucination apart from reality is an amalgam of self-certainty, self-reflection, executive functions, and memories.⁶² Apparently, the scope far exceeds those studied in iatrogenic anemia;

what we can assume from the phenomenon in this case is that said cognitive functions (and responsible brain regions) are sensitive to hypoxemia. Nevertheless, ascertaining whether schizophrenia relapses can occur from transient cognitive decline from anemia requires further studies. Moreover, whether the brain's reactive or autoregulatory changes toward anemia (vascular, metabolic, hormonal, etc) contribute to risk or protection of relapses also requires further studies.

There is also a paucity of reports of anemia triggering relapse in cases of established psychiatric illnesses other than schizophrenia. Generalizing about this suggested mechanism of impaired cognitive function, clinicians encountering anemia may also look for emergence or relapse of other psychiatric diseases in which cognitive dysfunctions play substantial roles, such as depression, anxiety, and neurocognitive disorders.^{63–68} These conditions involve different cognitive domains as opposed to schizophrenia which may become impaired at a different severity of anemia.

For clinicians encountering relapses of schizophrenia or mood disorders, consideration should be given that anemia could be among the differential diagnoses. When occurring in concert with anemia, correction of anemia alone may resolve these psychiatric symptoms. Psychotropic medication adjustment may be unnecessary or only used judiciously for temporary stabilization of severe agitation. For psychiatrists, monitoring for signs of anemia, surveillance of blood counts when indicated, and prompt referral for hematological treatment will serve to complement biological aspects of psychiatric care.

Limitations

There were limitations in the definite diagnosis of anemia in the present case. The onset was presumed from somatic symptom history. The comorbid diagnosis of pure red cell aplasia was presumptive and lacked bone marrow study data. Although much rarer than zidovudine-induced one, HIV-related immunohemolysis in well controlled cases remained a possibility. The possible underlying HIV infection-associated brain pathology and subsequent predisposition toward triggers of decompensation might differentiate the case from typical schizophrenia patients.

Conclusion

A case of a stable schizophrenia patient who experienced relapse following severe anemia from zidovudine-related immunohemolysis and red cell aplasia was described. Schizophrenia symptoms promptly returned to baseline following transfusion and did not necessitate changes in psychiatric medication; symptoms remained stable over 6 months of follow-ups. Clinicians encountering schizophrenia relapses may also evaluate their patients for anemia; such psychoses may be promptly reversible as reported in this case.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Vieth JT, Lane DR. Anemia. *Hematol Oncol Clin North Am*. 2017;31(6):1045–1060. doi:10.1016/j.hoc.2017.08.008
2. Beghè C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. *Am J Med*. 2004;116(Suppl 7A):3S–10S. doi:10.1016/j.amjmed.2003.12.009
3. Lucca U, Tettamanti M, Mosconi P, et al. Association of mild anemia with cognitive, functional, mood and quality of life outcomes in the elderly: the “Health and Anemia” study. *PLoS One*. 2008;3(4):e1920. doi:10.1371/journal.pone.0001920
4. Agrawal S, Kumar S, Ingole V, et al. Does anemia affects cognitive functions in neurologically intact adult patients: two year cross sectional study at rural tertiary care hospital. *J Fam Med Prim Care*. 2019;8(9):3005–3008. doi:10.4103/jfmpe.jfmpe_599_19
5. Nazari M, Mohammadnejad E, Dalvand S, Ghanei Gheshlagh R. Prevalence of iron deficiency anemia in Iranian children under 6 years of age: a systematic review and meta-analysis. *J Blood Med*. 2019;10:111–117. doi:10.2147/JBM.S196102
6. Kang SY, Kim HB, Sunwoo S. Association between anemia and maternal depression: a systematic review and meta-analysis. *J Psychiatr Res*. 2020;122:88–96. doi:10.1016/j.jpsychires.2020.01.001
7. Hector M, Burton JR. What are the psychiatric manifestations of vitamin B12 deficiency? *J Am Geriatr Soc*. 1988;36(12):1105–1112. doi:10.1111/j.1532-5415.1988.tb04397.x
8. Lachner C, Steinle NI, Regenold WT. The neuropsychiatry of vitamin B12 deficiency in elderly patients. *J Neuropsychiatry Clin Neurosci*. 2012;24(1):5–15. doi:10.1176/appi.neuropsych.11020052
9. Huemer M, Baumgartner MR. The clinical presentation of cobalamin-related disorders: from acquired deficiencies to inborn errors of absorption and intracellular pathways. *J Inherit Metab Dis*. 2019;42(4):686–705. doi:10.1002/jimd.12012
10. Hutto BR. Folate and cobalamin in psychiatric illness. *Compr Psychiatry*. 1997;38(6):305–314. doi:10.1016/S0010-440X(97)90925-1

11. Kale A, Naphade N, Sapkale S, et al. Reduced folic acid, vitamin B12 and docosahexaenoic acid and increased homocysteine and cortisol in never-medicated schizophrenia patients: implications for altered one-carbon metabolism. *Psychiatry Res.* 2010;175(1–2):47–53. doi:10.1016/j.psychres.2009.01.013
12. Wiser M, Levkowitz Y, Neuman M, Yehuda S. Decrease of serum iron in acutely psychotic schizophrenic patients. *Int J Neurosci.* 1994;78(1–2):49–52. doi:10.3109/00207459408986045
13. Shah HE, Bhawnani N, Ethirajulu A, et al. Iron deficiency-induced changes in the hippocampus, corpus striatum, and monoamines levels that lead to anxiety, depression, sleep disorders, and psychotic disorders. *Cureus.* 2021. doi:10.7759/cureus.18138
14. Sadock BJ, Sadock VA, Ruiz P. *Kaplan & Sadock's Comprehensive Textbook of Psychiatry*. Tenth ed. Wolters Kluwer; 2017.
15. Wysokiński A, Szczepocka E. Red blood cells parameters in patients with acute schizophrenia, unipolar depression and bipolar disorder. *Psychiatr Danub.* 2018;30(3):323–330. doi:10.24869/psyd.2018.323
16. Dudley AW, Waddell CC. Crisis in sickle cell trait. *Hum Pathol.* 1991;22(6):616–618. doi:10.1016/0046-8177(91)90242-H
17. Telen MJ, Roberts KB, Bartlett JA. HIV-associated autoimmune hemolytic anemia: report of a case and review of the literature. *J Acquir Immune Defic Syndr.* 1990;3(10):933–937.
18. Barry M, Howe JL, Back DJ, et al. Zidovudine pharmacokinetics in zidovudine-induced bone marrow toxicity. *Br J Clin Pharmacol.* 1994;37(1):7–12. doi:10.1111/j.1365-2125.1994.tb04231.x
19. Koduri PR, Singa P, Nikolinakos P. Autoimmune hemolytic anemia in patients infected with human immunodeficiency virus-1. *Am J Hematol.* 2002;70(2):174–176. doi:10.1002/ajh.10096
20. Balakrishnan A, Valsalan R, Sheshadri S, Pandit VR, Medep V, Agrawal RK. Zidovudine-induced reversible pure red cell aplasia. *Indian J Pharmacol.* 2010;42(3):189–191. doi:10.4103/0253-7613.66845
21. Insiripong S. HIV infection complicated by autoimmune hemolytic anemia (AIHA): a case report. *J Hematol Transfus Med.* 2017;27:159–163.
22. Iordache L, Launay O, Bouchaud O, et al. Autoimmune diseases in HIV-infected patients: 52 cases and literature review. *Autoimmun Rev.* 2014;13(8):850–857. doi:10.1016/j.autrev.2014.04.005
23. Kebede SS, Yalew A, Yesuf T, Melku M, Bambo GM, Woldu B. The magnitude and associated factors of immune hemolytic anemia among human immunodeficiency virus infected adults attending University of Gondar comprehensive specialized hospital north west Ethiopia 2021 GC, cross sectional study design. *PLoS One.* 2022;17(10):e0274464. doi:10.1371/journal.pone.0274464
24. Dogan M, Ozdemir O, Sal EA, et al. Psychotic disorder and extrapyramidal symptoms associated with vitamin B12 and folate deficiency. *J Trop Pediatr.* 2009;55(3):205–207. doi:10.1093/tropej/fmn112
25. Ende M, Klauber B, Gendel BR. Electric shock therapy of acute psychosis associated with pernicious anemia; report of a case. *Arch Neurol Psychiatry.* 1950;63(1):110–112. doi:10.1001/archneurpsyc.1950.02310190116009
26. Betts WC. The use of electroshock therapy in a psychosis associated with pernicious anemia. *N C Med J.* 1952;13(7):321–324.
27. Tripathi AK, Verma SP, Himanshu D. Acute psychosis: a presentation of cyanocobalamin deficiency megaloblastic anemia. *Indian J Hematol Blood Transfus.* 2010;26(3):99–100. doi:10.1007/s12288-010-0032-4
28. Payinda G, Hansen T. Vitamin B(12) deficiency manifested as psychosis without anemia. *Am J Psychiatry.* 2000;157(4):660–661. doi:10.1176/appi.ajp.157.4.660
29. Masalha R, Chudakov B, Morad M, Rudoy I, Volkov I, Wirguin I. Cobalamin-responsive psychosis as the sole manifestation of vitamin B12 deficiency. *Isr Med Assoc J.* 2001;3(9):701–703.
30. Silva B, Velosa A, Barahona-Corrêa JB. Reversible dementia, psychotic symptoms and epilepsy in a patient with vitamin B12 deficiency. *BMJ Case Rep.* 2019;12(5):e229044. doi:10.1136/bcr-2018-229044
31. Green R, Miller JW. Vitamin B12 deficiency. In: *Vitamins and Hormones*. Vol. 119. Elsevier; 2022:405–439.
32. Insel BJ, Schaefer CA, McKeague IW, Susser ES, Brown AS. Maternal iron deficiency and the risk of schizophrenia in offspring. *Arch Gen Psychiatry.* 2008;65(10):1136–1144. doi:10.1001/archpsyc.65.10.1136
33. Sørensen HJ, Nielsen PR, Pedersen CB, Mortensen PB. Association between prepartum maternal iron deficiency and offspring risk of schizophrenia: population-based cohort study with linkage of Danish national registers. *Schizophr Bull.* 2011;37(5):982–987. doi:10.1093/schbul/sbp167
34. Maxwell AM, Rao RB. Perinatal iron deficiency as an early risk factor for schizophrenia. *Nutr Neurosci.* 2022;25(10):2218–2227. doi:10.1080/1028415X.2021.1943996
35. Pisansky MT, Wickham RJ, Su J, et al. Iron deficiency with or without anemia impairs prepulse inhibition of the startle reflex: iron deficiency impairs prepulse inhibition. *Hippocampus.* 2013;23(10):952–962. doi:10.1002/hipo.22151
36. Kim SW, Stewart R, Park WY, et al. Latent iron deficiency as a marker of negative symptoms in patients with first-episode schizophrenia spectrum disorder. *Nutrients.* 2018;10(11):1707. doi:10.3390/nu10111707
37. Sui YV, McKenna F, Bertisch H, et al. Decreased basal ganglia and thalamic iron in early psychotic spectrum disorders are associated with increased psychotic and schizotypal symptoms. *Mol Psychiatry.* 2022;27(12):5144–5153. doi:10.1038/s41380-022-01740-2
38. Egyed M, Rajnics P, Kollár B, Sinkó J, Zsoldos E, Repa I. Severe hemolytic anemia and acute psychosis caused by *Clostridium perfringens* sepsis. *Med Sci Monit.* 2008;14(3):CS13–16.
39. Vucelić V, Stancić V, Ledinsky M, et al. Combined megaloblastic and immunohemolytic anemia associated—a case report. *Acta Clin Croat.* 2008;47(4):239–243.
40. Bentall RP, Slade PD. Reality testing and auditory hallucinations: a signal detection analysis. *Br J Clin Psychol.* 1985;24(3):159–169. doi:10.1111/j.2044-8260.1985.tb01331.x
41. McDannald M, Schoenbaum G. Toward a model of impaired reality testing in rats. *Schizophr Bull.* 2009;35(4):664–667. doi:10.1093/schbul/sbp050
42. McDannald MA, Whitt JP, Calhoon GG, et al. Impaired reality testing in an animal model of schizophrenia. *Biol Psychiatry.* 2011;70(12):1122–1126. doi:10.1016/j.biopsych.2011.06.014
43. Cannon TD. How schizophrenia develops: cognitive and brain mechanisms underlying onset of psychosis. *Trends Cogn Sci.* 2015;19(12):744–756. doi:10.1016/j.tics.2015.09.009
44. Weiskopf RB, Kramer JH, Viele M, et al. Acute severe isovolemic anemia impairs cognitive function and memory in humans. *Anesthesiology.* 2000;92(6):1646–1652. doi:10.1097/00000542-200006000-00023
45. Weiskopf RB, Feiner J, Hopf HW, et al. Oxygen reverses deficits of cognitive function and memory and increased heart rate induced by acute severe isovolemic anemia. *Anesthesiology.* 2002;96(4):871–877. doi:10.1097/00000542-200204000-00014

46. Weiskopf RB, Toy P, Hopf HW, et al. Acute isovolemic anemia impairs central processing as determined by P300 latency. *Clin Neurophysiol.* 2005;116(5):1028–1032. doi:10.1016/j.clinph.2004.12.009
47. Allali S, Brousse V, Sacri AS, Chalumeau M, de Montalembert M. Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. *Expert Rev Hematol.* 2017;10(11):1023–1028. doi:10.1080/17474086.2017.1354696
48. Choi S, O'Neil SH, Joshi AA, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* 2019;94(10):1055–1065. doi:10.1002/ajh.25570
49. Drew DA, Weiner DE, Sarnak MJ. Cognitive impairment in CKD: pathophysiology, management, and prevention. *Am J Kidney Dis.* 2019;74(6):782–790. doi:10.1053/j.ajkd.2019.05.017
50. Prussien KV, Compas BE, Siciliano RE, et al. Cerebral hemodynamics and executive function in sickle cell anemia. *Stroke.* 2021;52(5):1830–1834. doi:10.1161/STROKEAHA.120.032741
51. Limpawattana P, Juntararungtong T, Teawtrakul N, et al. Cognitive impairment in thalassemia and associated factors. *Dement Geriatr Cogn Disord.* 2022;51(2):128–134. doi:10.1159/000522655
52. Cunningham RS. Anemia in the oncology patient: cognitive function and cancer. *Cancer Nurs.* 2003;26:38S??42S. doi:10.1097/00002820-200312001-00009
53. Kung WM, Yuan SP, Lin MS, et al. Anemia and the risk of cognitive impairment: an updated systematic review and meta-analysis. *Brain Sci.* 2021;11(6):777. doi:10.3390/brainsci11060777
54. Adomaitienė V, Jaras A, Lauciūtė E, Liugailaitė S, Dambrauskienė K. P03-16 - causes and first symptoms of schizophrenia relapse. *Eur psychiatr.* 2010;25(S1):1.
55. Emsley R, Chiliza B, Asmal L, Harvey BH. The nature of relapse in schizophrenia. *BMC Psychiatry.* 2013;13(1):50. doi:10.1186/1471-244X-13-50
56. Momtazmanesh S, Zare-Shahabadi A, Rezaei N. Cytokine alterations in schizophrenia: an updated review. *Front Psychiatry.* 2019;10:892. doi:10.3389/fpsy.2019.00892
57. Pothimas N, Tungpunkom P, Chanprasit C, Kitsumban V, Cross-sectional A. Study of factors predicting relapse in people with schizophrenia. *PRIJNR.* 2020;24(4):448–459.
58. Fond G, Lançon C, Korchia T, Auquier P, Boyer L. The role of inflammation in the treatment of schizophrenia. *Front Psychiatry.* 2020;11:160. doi:10.3389/fpsy.2020.00160
59. Sahpolat M, Ayar D, Ari M, Karaman MA. Elevated monocyte to high-density lipoprotein ratios as an inflammation markers for schizophrenia patients. *Clin Psychopharmacol Neurosci.* 2021;19(1):112–116. doi:10.9758/cpn.2021.19.1.112
60. Parksepp M, Haring L, Kilk K, et al. A marked low-grade inflammation and a significant deterioration in metabolic status in first-episode schizophrenia: a five-year follow-up study. *Metabolites.* 2022;12(10):983. doi:10.3390/metabo12100983
61. Portilla I, Reus S, León R, et al. Neurocognitive impairment in well-controlled HIV-infected patients: a cross-sectional study. *AIDS Res Hum Retroviruses.* 2019;35(7):634–641. doi:10.1089/aid.2018.0279
62. Ohmuro N, Katsura M, Obara C, et al. The relationship between cognitive insight and cognitive performance among individuals with at-risk mental state for developing psychosis. *Schizophr Res.* 2018;192:281–286. doi:10.1016/j.schres.2017.04.031
63. Daleiden E. An information-processing perspective on childhood anxiety. *Clin Psychol Rev.* 1997;17(4):407–429. doi:10.1016/S0272-7358(97)00010-X
64. Castaneda AE, Tuulio-Henriksson A, Marttunen M, Suvisaari J, Lönnqvist J. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J Affect Disord.* 2008;106(1–2):1–27. doi:10.1016/j.jad.2007.06.006
65. Murrough JW, Iacoviello B, Neumeister A, Charney DS, Iosifescu DV. Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. *Neurobiol Learn Mem.* 2011;96(4):553–563. doi:10.1016/j.nlm.2011.06.006
66. Carvalho A, Miskowiak K, Hyphantis T, et al. Cognitive dysfunction in depression – pathophysiology and novel targets. *CNSDDT.* 2015;13(10):1819–1835. doi:10.2174/1871527313666141130203627
67. Hur J, Stockbridge MD, Fox AS, Shackman AJ. Dispositional negativity, cognition, and anxiety disorders: an integrative translational neuroscience framework. In: *Progress in Brain Research.* Vol. 247. Elsevier; 2019:375–436.
68. Shi R, Sharpe L, Abbott M. A meta-analysis of the relationship between anxiety and attentional control. *Clin Psychol Rev.* 2019;72:101754. doi:10.1016/j.cpr.2019.101754

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Safe Blood Donation from Donors Using Antihypertensive Medication. A Multi-Center Retrospective Quality Study from South-East Norway

Kathrine M Neuman Johnsen, Karin Magnussen, Christian Erstad, Sadaf Nabi Bhatti & Lise Sofie H Nissen-Meyer

To cite this article: Kathrine M Neuman Johnsen, Karin Magnussen, Christian Erstad, Sadaf Nabi Bhatti & Lise Sofie H Nissen-Meyer (2023) Safe Blood Donation from Donors Using Antihypertensive Medication. A Multi-Center Retrospective Quality Study from South-East Norway, Journal of Blood Medicine, , 337-343, DOI: [10.2147/JBM.S390609](https://doi.org/10.2147/JBM.S390609)

To link to this article: <https://doi.org/10.2147/JBM.S390609>



© 2023 Johnsen et al.



Published online: 03 May 2023.



Submit your article to this journal



Article views: 352



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Safe Blood Donation from Donors Using Antihypertensive Medication. A Multi-Center Retrospective Quality Study from South-East Norway

Kathrine M Neuman Johnsen^{1,2}, Karin Magnussen³, Christian Erstad³, Sadaf Nabi Bhatti⁴, Lise Sofie H Nissen-Meyer²

¹Center for Laboratory Medicine and Blood Bank, Østfold Hospital Trust, Grålum, Norway; ²Department of Immunology, Oslo University Hospital, Oslo, Norway; ³Department of Immunology and Transfusion Medicine, Innlandet Hospital Trust, Lillehammer, Norway; ⁴Department of Immunology and Transfusion Medicine, Akershus University Hospital, Lørenskog, Norway

Correspondence: Kathrine M Neuman Johnsen, Østfold Hospital Trust, P.O. Box 300, Grålum, 1714, Norway, Tel +47 669868410; +4796090859, Email Kathrine.M.Neuman.Johnsen@so-hf.no

Purpose: In Norway, blood donors using antihypertensive medication were deferred until 2015. Following revision of the national directive, these donors could be allowed, providing stable dose for at least 3 months, adequate blood pressure control and no adverse effects caused by the therapy. The new practice was evaluated by a quality study where the major aim was to establish whether donations from blood donors on antihypertensive medication pose a risk to the donor. The risk was assessed by counting the number and categorizing the adverse events related to blood donation. In addition, the quantitative effect of including these donors was calculated.

Subjects and Methods: In this retrospective quality study, blood donors on antihypertensive therapy were recruited from four different blood centers to fill out a questionnaire. A total of 265 donors answered questions regarding their health status, type of medication used, and adverse events connected to blood donation both before and after starting the therapy.

Results: No severe adverse events were observed in donors on antihypertensive medications. The amount of mild adverse events, as exhibited by only 7 persons (0.46%) in this donor population, was the same as for donors without hypertensive treatment.

Conclusion: Blood donation from persons on antihypertensive therapy poses no extra risk of severe adverse events, given the use of screening criteria to identify and bleed only low-risk donors.

Keywords: donor safety, hemovigilance, hypertension, deferral

Introduction

Donors with moderately elevated blood pressure are allowed to donate blood as long as blood pressure measurements performed in the blood center are below the limit (180/100 mmHg). Despite it being counter-intuitive to defer them from blood donation following the start of relevant medical therapy, Norwegian blood banks generally deferred blood donors on antihypertensive treatment until 2015. The national guidelines allowed only donors using diuretics (thiazides) to donate blood without further assessment, and all other antihypertensive medications depended upon the doctors' evaluation and approval. Without guidelines to instruct the physicians about the safety of antihypertensive medications in the context of blood donation, each doctor was left to make her own decision.¹ This led to different practices in different blood donation centers. The donors on antihypertensive medication were generally deferred due to the principle that blood donors should be completely healthy. In addition, since the physiological mechanisms which compensate for donation-induced hypotension were perceived to be inhibited by the therapy, there was a concern for donor safety. The assumption was made that rapid blood loss due to donation could increase the risk of hypotensive reactions, ie, dizziness

and syncope. As a result, a significant number of otherwise healthy and highly motivated donors were deferred. Although the evidence in the literature is poor,² a few reports have indicated that blood donation from donors using antihypertensive therapy is unproblematic.^{3–5}

Therefore, the responsible medical consultants in four different blood centers of southeast Norway decided to adjust the local guidelines, to allow donors using antihypertensive drugs to donate blood from 2015. To help us choose which donors to accept for donation, we established a set of guidelines (Box 1). In addition, the following safety rules were generally applied: Donors using angiotensin II receptor antagonists (AIIAs) and angiotensin converting enzyme inhibitors (ACEi) were deferred from donation by apheresis, due to possible risk of anaphylaxis and hypotension mediated by the bradykinin axis.⁶ These donors were, however, allowed to donate whole blood. Donors using calcium channel inhibitors were checked for edemas and pulse irregularities before and after donation, these being the most common side effects of antihypertensive drugs. Donors treated with alpha and/or beta receptor blockers have been deferred both before and after the 2015 revision, mostly because these medications are not first-line therapeutics for primary hypertension.

Two years after the guideline changes, we conducted a follow-up quality study with the following aims: To investigate whether it is safe for the donors who use antihypertensive medication to donate blood and to see if these types of donors experience adverse events more frequently compared to other donors. We also wanted to validate the selection criteria for donors on antihypertensive medication and estimate the number of extra donors and donations gained by acceptance of these donors.

Materials and Methods

Information from 265 donors was collected from four blood centers in the southeast part of Norway. The centers were located in Oslo, Akershus, Innlandet og Østfold counties. Blood donation data for the participating blood centers are shown in Table 1. Data collection was performed for 3–4 months at each site, whereafter de-identified data was combined and analyzed using Microsoft Excel.

All donors reporting relevant medication were given the opportunity to participate in the study. Established donors on antihypertensive medication who were allowed to donate following the guidelines (Box 1) had been given a special code in the blood bank laboratory system that enabled us to locate and address them for inclusion. In addition, new donors using this type of medication who were admitted during the inclusion period were also invited to participate. Eleven donors did not wish to attend. One donor was deferred and excluded from further analysis due to treatment with the beta receptor blocker bisoprolol.

Each donor was asked to complete a questionnaire we had developed for this study.

We made queries regarding health status including additional diagnoses, type of medications used, and efficacy of and side effects to antihypertensive treatment. We also requested information about any adverse reactions related to blood donation while on medication and asked the donors to estimate their extra fluid intake in connection with donations. Signed informed consent from each donor was obtained and stored along with the above mentioned questionnaire. The study complies with the Declaration of Helsinki and was approved as a quality study by the data protection officer at Oslo University Hospital (2017-6600) and by equivalent bodies in the other hospitals.

Box 1 Local Guidelines for Blood Donation from Donors Using Antihypertensive Medication

- Only donors with essential/primary hypertension were eligible for donation.
- Donors with hypertension secondary to other medical conditions were deferred.
- The donor should have received a stable treatment dose for at least 3 months.
- Any side effects of hypertensive medications, like dizziness, orthostatic hypotension or edemas, led to deferral.
- The donor's blood pressure, measured in the blood center, had to be within normal range.
- Donors taking one or two types of antihypertensive drugs were allowed. Acceptable combination preparations included ACE inhibitor or angiotensin II receptor antagonists combined with thiazides or calcium channel blockers.
- Donors on antihypertensive medication were accepted for blood donation regardless of blood donation history.
- Initially, the donors were interviewed by a blood center medical consultant before acceptance for donation.
- Every donor on antihypertensive medication was treated with extra vigilance during and after donation.

Table I Blood Donation Data from the Participating Blood Centres

Blood Centre	Total Number of Donations 2017	Number of Donors Included in this Study
Akershus	13,949	55
Innlandet	11,402	29
Østfold	18,027	50
Oslo	34,346	130

Results

A total of 264 donors who responded to the survey and gave their consent to data collection used one of the approved medications. The demographic characteristics of the participating donors are shown in Figure 1. The average age of the participants was 55 years (170 men and 94 women). Most of the donors were prescribed antihypertensive medication due to a diagnosis of essential hypertension. Of these donors, 70% were treated in primary health care. Other causes for hypertension, eg, stress and obesity were noted in 13% of the participants. Analysis showed that two of the donors included were taking antihypertensive medication for migraines. While these persons were not within the initial scope of the study, the novelty of acceptance of antihypertensive medication in this instance had overshadowed the indication. Since they had consented to participation, we decided to include the information from these donors in the analysis.

None of the donors had secondary hypertension or serious underlying conditions. Before starting the antihypertensive medication, 85% of the donors had donated a median of 27 times (variation: 0–319; Table 2), with a total of 9661 donations.

Figure 2 shows the distribution of different antihypertensive medication used by the donors in the study. Angiotensin II (AII) receptor antagonists in monotherapy were found in 54% of donors. More men than women were using angiotensin-converting enzyme (ACE)-inhibitors, whereas more women than men were using Ca²⁺-channel inhibitors. Of the 80 (30%) using combination therapies, as many as 67 (5 out of 6) were using combinations containing angiotensin II receptor antagonists. The median treatment length was 14 months (range 1 to 414).

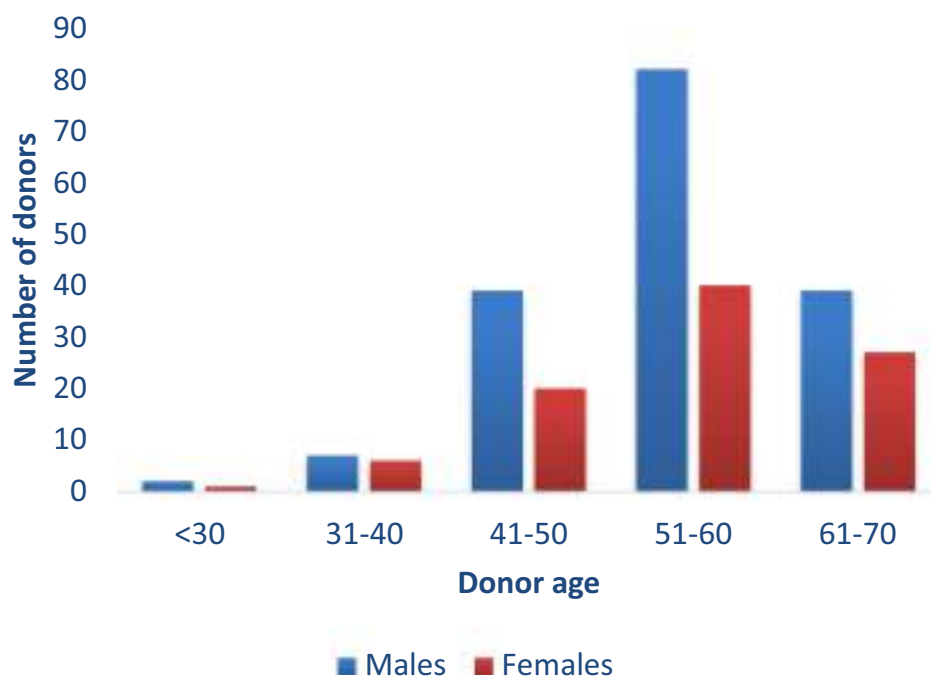


Figure 1 Demographics of the donors included in the study (n=264). Histogram showing the distribution of male (blue columns) and female (red columns) donors in groups based on age (horizontal axis).

Table 2 Summary of Donor Characteristics

Median treatment length (months; range)	14 (1*–414)
Median number of donations before treatment (range)	27 (0–319)
Total number of donations before treatment (all donors)	9661
Median number of donations on treatment (range)	5 (1–21)
Total number of donations on treatment	1527
Number of AEs within BE (%)	2 (0.13)
Number of AEs outside BE (%)	5 (0.33)
Total number of AEs on treatment (%)	7 (0.46)

Notes: *In spite of the guidelines, some donors reported shorter treatment time before starting blood donation. Types of AE: within BE: dizziness (2); outside BE: dyspnea upon activity (2) and dizziness (3).

Abbreviations: AE, adverse events; BE, blood establishments.

A total of 68% of the donors had no other illnesses and used no other therapies, whereas 84 donors (32%) reported using additional medications. In this group, 30% used cholesterol-reducing prescriptions. Additional medications in the form of anti-allergic or anti-asthmatic prescriptions were present in 25% of donors, 10% with thyroid substitution and 10% used proton pump inhibitors. Only 5% reported other hormonal substitution therapies and 10% used more than one medication (of which one is cholesterol-reducing).

Correspondingly, 63 donors (24%) reported to have other conditions: of those, 40% had allergy/asthma, 11% were treated for hypercholesterolemia, 16% had thyroid disorders, 11% suffered from gastric reflux, 5% had migraine, and 5% reported having uric acid arthritis.

This study revealed that, following the changed guidelines, the included donors had contributed a total of 1527 units of blood (ranging from 1 to 21), yielding median 5 units per donor, as shown in Table 2.

Following 7 of these donations, the donors reported adverse events (0.46%), none of which was considered severe (Table 2). Two events of dizziness occurred before the donor had left the blood center, whereas two episodes of dyspnea upon activity and three episodes of dizziness were reported from outside the blood establishment following donation. In the same period, the frequency of mild adverse events in 5645 donations from donors without antihypertensive medication was 0.44% in comparison.

Donors showed excellent compliance following advice given by the blood center staff. More than 90% drank 0.5 liters or more of liquids upon donation, and mean liquid intake was 0.62 liters. Three of the donors who experienced adverse effects had been drinking 0.25–0.3 liters, and all three were female.

The distribution of blood types among the participating donors is shown in Figure 3.

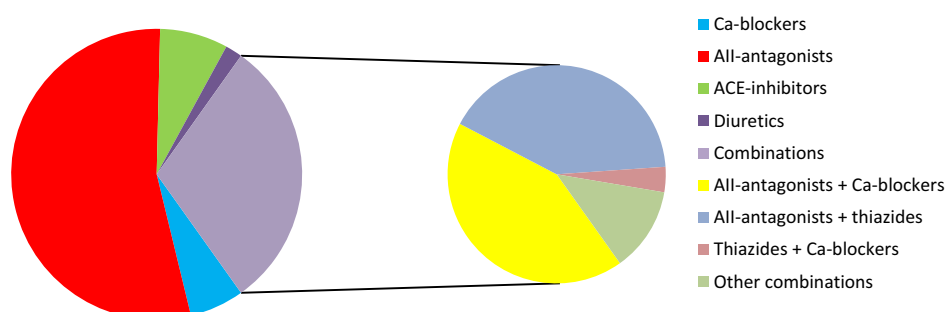


Figure 2 Distribution of different antihypertensive medication used by the donors (n=264). For the 80 donors using combination therapies, the distribution of these combinations is shown to the right.

Abbreviations: All antagonists, angiotensin II receptor antagonists; ACE-inhibitors, angiotensin converting enzyme inhibitors; Ca blockers, calcium channel blockers.

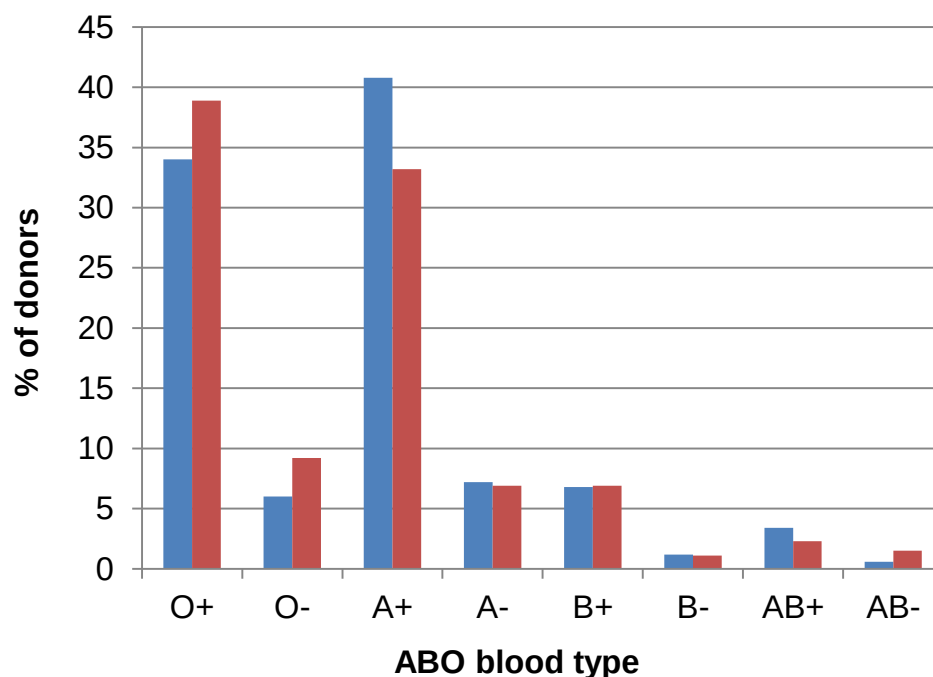


Figure 3 Distribution of ABO/Rh (D) blood types among the blood donors included in this study. Blue columns indicate the mean proportion of each blood type in the general Norwegian population, and red columns show the distribution of these blood types in the current study.

Discussion

Large studies of whole blood donations have determined predictors of adverse reactions following blood donation to be having a donor blood volume of less than 3500 mL, young age and first-time donation.⁷ In more than 400 000 allogeneic donations, the reaction prevalence was 1.43%. Blood pressure or pulse frequency was not associated with increased risk for reactions.

Our findings showed a similar number of mild adverse events (AE) in donors on antihypertensive medication (0.46%), compared to the general donor population in this period (0.44%). In line with data discussed above, three of the AEs in this study occurred in females who used to drink less than the average volume. This frequency is, however, low compared to previous reports of donor reactions following whole blood donation, where an overall reaction prevalence ranged from 1.4 to 5%.^{7,8} The Norwegian Hemovigilance program has no relevant data for comparison locally since only data on severe adverse events is reported (0.05% in 2016 and 2017). However, 0.65% of whole blood donors in Saudi-Arabia experienced mild adverse events including dizziness.⁹ Our medicated donors reported no severe adverse events. We postulate that this may be attributed to previous donation experience, good compliance with regard to drinking fluids during donation, and extra attention from the staff.

In our study population of blood donors treated for hypertension, the number of male donors was almost twice the number of female donors. This contrasts with the general Norwegian blood donor population. In Oslo Blood Center, females constitute 57% of all donors.¹⁰ However, the numbers reflect the age and gender distribution of hypertension treatment in the general population, being a condition of increased age and more common in men.¹¹ Norwegian primary care practitioners diagnose and treat hypertension in line with national recommendations.¹² ACE-inhibitors, angiotensin II receptor antagonists, diuretics and calcium channel blockers are used for primary prophylactic treatment, whereas alpha- and beta blockers are considered for secondary prophylaxis following the first incidents of heart disease. Therefore, the distribution of medication in our blood donors reflects current prescription practices. In addition, there is a selection bias since alpha and beta blockers are still not generally accepted in Norwegian blood donors.

In our study, 32% of the donors using antihypertensive medication also used other medication, and 24% reported having other medical conditions. Thus, the idea of the blood donor being “completely healthy” is nowadays less valid than before. In recent years, several eligibility criteria have been revised to increase the availability of blood donors, both due to lack of new

donors but also to prevent unnecessary loss of established donors. It has been suggested that several of the traditional causes for blood donor deferrals are not evidence-based but on consensus and “better safe than sorry”-arguments.¹³

As shown in Figure 3, the blood-type distribution of our medicated population largely reflects the distribution in the general Norwegian population. Although the sample size is small, this indicates that the acceptance of blood donors treated with antihypertensive medication is general and not restricted to the most-needed blood types in the blood center. Thus, the changed rule represents an improved donation policy. Furthermore, the donors on antihypertensive medication provided a much-welcomed addition of blood products (Table 2), from earlier unexploited resources, which will increase in the future. The donors in our study are reliable and highly motivated, and they have reached an age which is associated with low numbers of deferrals for the most common reasons seen in younger donors.

Conclusion

Our study supports the continuous acceptance of blood donors despite anti-hypertensive drug therapy, as they do not experience more frequent AEs than other donors. In part, this can be explained by this group having higher baseline blood pressures than other donors, their experience from previous donations, and good compliance with advice offered by the blood bank personnel.

The inclusion of this group of blood donors has led to a welcome extra supply of whole blood units to our blood centers.

Acknowledgments

The authors thank all the blood donors who contributed, as well as the personnel in the blood centers for assistance in recruiting and handling the donors.

Disclosure

The authors report no conflicts of interest in this work.

References

1. HelseDirektoratet [Norwegian Directorate of Health]. Veileder for transfusjonstjenesten i Norge, utgave 7.3 [Guidelines for the Blood Transfusion Services in Norway, edition 7.3]. 75. Available from: <https://www.helsedirektoratet.no/veiledere/transfusjonstjenesten-i-norge-utgave-7.3>. Accessed December 27, 2022. Norwegian.
2. Stainsby D, Brunskill S, Chapman CE, Dorée C, Stanworth S. Safety of blood donation from individuals with treated hypertension or non-insulin dependent type 2 diabetes – a systematic review. *Vox Sang*. 2010;98:431–440. doi:10.1111/j.1423-0410.2009.01275.x
3. Thomas SHL, Weston Smith S, Slater NGP, Pearson TC, Treacher DF. The haemodynamic responses to venesection and the effects of cardiovascular disease. *Clin lab Haematol*. 1992;14:201–208. doi:10.1111/j.1365-2257.1992.tb00366.x
4. Weisbach V, Schnabel L, Zimmermann R, Zingsem J, Eckstein R. A pilot study of continuous ambulatory monitoring of blood pressure in repeated preoperative autologous blood donation. *Transfusion*. 2006;46:934–941. doi:10.1111/j.1537-2995.2006.00825.x
5. Pisciotto P, Sataro P, Blumberg N. Incidence of adverse reactions in blood donors taking antihypertensive medications. *Transfusion*. 1982;22(6):530–531. doi:10.1046/j.1537-2995.1982.22683068620.x
6. Jurakić Tončić R, Marinović B, Lipozenčić J. Nonallergic hypersensitivity to nonsteroidal antiinflammatory drugs, angiotensin-converting enzyme inhibitors, radiocontrast media, local anesthetics, volume substitutes and medications used in general anesthesia. *Acta Dermatovenereol Croat*. 2009;17(1):54–69.
7. Wiltbank TB, Giordano GF, Kamel H, Tomasulo P, Custer B. Faint and prefaint reactions in whole-blood donors: an analysis of predonation measurements and their predictive value. *Transfusion*. 2008;48:1799–1808. doi:10.1111/j.1537-2995.2008.01745.x
8. Newman BH. Donor reactions and injuries from whole blood donation. *Transfus Med Rev*. 1997;11:64–75. doi:10.1016/S0887-7963(97)80011-9
9. Almutairi H, Salam M, Alajlan A, Wani F, Al-Shammari B, Al-Surimi K. Incidence, predictors and severity of adverse events among whole blood donors. *PLoS One*. 2017;12(7):e0179831. doi:10.1371/journal.pone.0179831
10. Nissen-Meyer LSH, Heier HE. Probability to be a blood donor in Oslo depending on geographical factors. Abstract, 35th International Congress of the ISBT, Toronto, Canada, June 2–6, 2018. *Vox Sang*. 2018;113(Suppl 1):5–347. doi:10.1111/vox.12658
11. Klouman M, Åsberg A, Widerøe T-E. Blood pressure level in a Norwegian population – the significance of heritage and lifestyle. *Tidsskr nor Lægeforen*. 2011;131:1185–1189. doi:10.4045/tidsskr.10.0486
12. HelseDirektoratet [Norwegian Directorate of Health]. Legemiddelbehandling av høyt blodtrykk [Pharmacologic antihypertensive therapy]. Available from: <https://www.helsedirektoratet.no/retningslinjer/forebygging-av-hjerte-og-karsykdom/legemidler-ved-primaerforebygging-av-hjerte-og-karsykdom/legemiddelbehandling-av-hoyt-blodtrykk>. Accessed December 27, 2022. Norwegian.
13. Eder A, Goldman M, Rossmann S, Waxman D, Bianco C. Selection criteria to protect the blood donor in North America and Europe: past (dogma), present (evidence), and future (hemovigilance). *Transfus Med Rev*. 2009;23(3):205–220. doi:10.1016/j.tmr.2009.03.003

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



A Single Center Study Investigating Clinical Outcomes of Testing for Multiple Myeloma and Immune Deficiency at Low Globulin Levels

Indra Ramasamy

To cite this article: Indra Ramasamy (2023) A Single Center Study Investigating Clinical Outcomes of Testing for Multiple Myeloma and Immune Deficiency at Low Globulin Levels, Journal of Blood Medicine, , 345-358, DOI: [10.2147/JBM.S409234](https://doi.org/10.2147/JBM.S409234)

To link to this article: <https://doi.org/10.2147/JBM.S409234>



© 2023 Ramasamy.



Published online: 30 May 2023.



Submit your article to this journal



Article views: 323



View related articles



View Crossmark data

A Single Center Study Investigating Clinical Outcomes of Testing for Multiple Myeloma and Immune Deficiency at Low Globulin Levels

Indra Ramasamy 

Department of Blood Sciences, Worcester Royal Hospital, Worcester, UK

Correspondence: Indra Ramasamy, Worcester Royal Hospital, Charles Hastings Way, Worcester, WR5 1DD, United Kingdom, Email indrar@ozemail.com.au

Background: Both primary (e.g. common variable immune deficiency, CVID) and secondary immune deficiency as well as multiple myeloma (MM) require medical intervention and treatment delay can exacerbate morbidity. This study investigated the potential importance of low levels of calculated globulin to detect immune deficiency and MM associated with immunoparesis (light chain, non-secretory MM).

Methods: One hundred and thirty-nine patient serum samples from community physicians and outpatient clinics for liver function tests with low calculated globulin (<16 g/L, RR 18–37 g/L) levels were screened for immunoglobulins and protein electrophoresis. Further, 110 patients with globulin levels ≤ 16 g/L with screening for immunoglobulin levels and protein electrophoresis, requested through routine clinical care, were included in the analysis.

Results: Approximately 47% of patients in this cohort had secondary antibody deficiency as a result of hematological malignancy. Secondary iatrogenic (immunosuppressants, antiepileptic drugs) immune deficiency was detected in 20% of patients and a significant percentage of the patients were found by reflex testing at globulin levels <16 g/L. During the study period the screening detected new light chain and non-secretory MM in 2.2% of patients. Three patients with CVID and six patients with light chain myeloma were previously detected by screening, consequently alerting clinicians and reducing delay in treatment. A further 23% with several co-morbid conditions showed unexpected hypogammaglobulinemia; in this category, the study identified a subgroup that required further investigation.

Conclusion: Investigation of low globulin levels detects patients with primary and secondary immune deficiency and MM. Optimizing treatment for decreased immunoglobulins in patients with other clinical co-morbidities may require increased clinician awareness and watchful clinical and laboratory assessment.

Keywords: calculated globulin, primary immune deficiency, common variable immune deficiency, secondary immune deficiency, light chain myeloma, non-secretory myeloma

Highlights

- We investigated the value of low globulin levels in the diagnosis of immune deficiency and multiple myeloma in a routine clinical laboratory.
- Low globulin levels identified patients with (i) immune deficiency, (ii) multiple myeloma, (iii) iatrogenic secondary immune deficiency (SID), (iv) SID in hematological malignancies and (v) idiopathic hypogammaglobulinemia.
- Patients with hematological malignancies were more likely to be monitored for SID than those on antiepileptic or immunosuppressant treatment.
- A subgroup of patients with unexpected hypogammaglobulinemia will benefit from further clinical evaluation.

Introduction

Calculated globulin (total protein-albumin) is part of the liver function test profile. The main use of calculated globulin is to determine paraprotein concentrations at high levels. There are few studies to investigate the use of globulin values below the reference range to detect both antibody deficiencies or new minor paraproteins associated with hypogammaglobulinemia (immunoparesis) e.g. light chain myeloma or non-secretory multiple myeloma. Both diagnoses are important because of the requirement for medical intervention and morbidity associated with delay in diagnosis which requires the use of health resources. Diagnostic and consequent treatment delay occurs when there is a failure to consider immune deficiency or multiple myeloma (MM) in patients with appropriate clinical presentation.

Approximately 15% of patients with myeloma have light chain myeloma. Light chain multiple myeloma has a more aggressive disease course and poor outcome. Renal failure, bone disease and amyloidosis appear to be more frequent in these patients.¹ Non-secretory multiple myeloma is a rare variant of multiple myeloma, because of its rarity there is a paucity of data regarding prognosis, though it appears to be less aggressive than light chain multiple myeloma.²

A comprehensive process for the detection of primary immune deficiency (PID) and secondary immune deficiency (SID) has not been explored. The genetics of PID is complex. The clinical presentation of PID, and one of the most common symptomatic primary immune deficiency disorders, common variable immune deficiency (CVID) is broad and may present as predominantly recurrent infections or may present with autoimmune, inflammatory conditions or malignancy.^{3,4} Secondary immune deficiency is more common than PID, and may present within a wider range of specialties and is associated with an increased risk of infection.⁵ Calculated globulin may be low in hypogammaglobulinemia. However, there is possibility for changes in other proteins to mask hypogammaglobulinemia e.g. acute phase proteins, such as complement and alpha1-antitrypsin can increase during inflammatory conditions causing an increase in calculated globulins. Transient hypogammaglobulinemia has been associated with respiratory infections; as the infection resolved, serum immunoglobulins returned to normal.⁶

Previous studies, in the UK, have indicated that calculated globulins facilitate early diagnosis of patients with unsuspected hypogammaglobulinemia and that the use of calculated globulin in screening for antibody deficiency may shorten the latency period for correct management of patients with immune deficiency.^{7,8} Both studies reviewed the detection of immune deficient patients using information from calculated globulin levels. An Australian study assessed the sensitivity and specificity of detection of hypogammaglobulinemia at low calculated globulin values, though the underlying clinical conditions for low immunoglobulin levels were not explored.⁹

New evidence suggests that patients with SID and PID are at greater risk of morbidity and mortality during pandemic periods such as during the Covid-19 pandemic.¹⁰ Identification of patients with immune deficiency at increased risk of co-morbidities during pandemics is relevant as public health guidelines to protect this group of vulnerable patients and to decrease adverse outcomes in PID and SID patients are required.

We report here on real life experience in the detection of immune deficiency or MM associated with immunoparesis in a district general hospital when protein electrophoresis and immunoglobulins were either requested by the physician or analysed as a result of opportunistic screening of serum samples with calculated globulin levels below the reference range. Unlike previous studies, this study evaluates the utility of investigating calculated globulins below the reference range for MM, PID and SID in a non-teaching hospital setting. In Worcestershire, UK, patients with globulin values outside the reference range are screened for protein electrophoresis and immunoglobulins. The screening service was started in 1977.¹¹ Worcester Royal Hospital (WRH) provides pathology services to a population of approximately 500,000 in both primary and secondary care. Opportunistic screening (or reflex testing) is part of the service offered by the laboratory, which in addition carries out protein electrophoresis requested through routine clinical care. In this cross-sectional observational study we investigated the advantages and disadvantages of low globulin values for the detection of both immune deficiency and MM with immunoparesis. We further review the cost implications to the health-care system of the protocol used at the WRH.

Materials and Methods

Selection of Patients and Primary Outcome

Serum samples sent for liver function tests were reflex tested for protein electrophoresis and immunoglobulins at globulin values <16 g/L (reference range 18–37 g/L) in patients ≥30 years of age. Calculated globulin values were

derived from liver function tests by subtracting albumin from total protein. Opportunistic screening (reflex testing) was performed if previous protein electrophoresis results were not available for a period of 12 months. Reflex testing was discretionary as hospital inpatients and outpatient patients from rheumatology, gastroenterology and non-hematologic oncology clinics were excluded from reflex testing since changes in immunoglobulin levels in these patients were likely to be due to other pathology or treatment and monitoring of patients was carried out by their individual specialties.

The study was designed as a retrospective cross-sectional observational study with data collected during the period January 1, 2019 to December 31, 2019. The laboratory information management system (WinPath, Clinisys, UK) enabled the reflex testing and automatic addition for immunoglobulin and protein electrophoresis analysis based on globulin levels, using a comprehensive WinPath code system. In addition, protein electrophoresis and immunoglobulin requested through routine clinical care in patients with globulin ≤ 16 g/L were incorporated in the study. Data on patient samples reflex tested for protein electrophoresis and immunoglobulins as well as patient samples with globulin ≤ 16 g/L with routine requests for immunoglobulin and protein electrophoresis were collected using the laboratory information management system. Discretionary reflex testing was carried out on all samples with globulin levels < 16 g/L every 12 months. The protocol was designed to include all patients with globulin levels ≤ 16 g/L with immunoglobulin and protein electrophoresis results during 2019 (Flow chart, Figure 1). Decrease in diagnostic delay was defined if the onset of symptoms preceded the diagnosis of myeloma/primary immune deficiency and the globulin levels were identified by reflex testing other than by routine requests for immunoglobulins. The primary end point of the study was the detection rate of both immune deficiency and MM as well as the diagnosis of patients with globulin values ≤ 16 g/L who were identified by both opportunistic screening and routine requests.

Analytical Methods

Patients with immunoglobulin levels below the limit of quantitation were assigned the lower limit of quantitation for calculation purposes. Total protein was measured using the biuret method and albumin using the bromocresol green method (Roche, UK). The analytical method for detection, typing and quantitation of paraprotein was capillary electrophoresis and immunosubtraction using Capillarys or immunofixation carried out using Hydragel (Sebia, UK). Serum free light chains were measured using Optilite (Binding site, UK) and immunoglobulins were measured on Cobas (Roche, UK).

Clinical comment was added if IgG < 6 g/L or if two or more immunoglobulin classes were below the reference range and a known cause of hypogammaglobulinemia was excluded. The clinical comment stated “exclude the following causes of low immunoglobulins, immunosuppressive drugs, antiepileptic drugs, hematological malignancies and renal/gastrointestinal protein loss. If the above causes excluded functional antibody studies suggested as a first step to investigate for primary immune deficiency states”. In patients < 30 years of age reflex testing for protein electrophoresis was excluded as MM was unlikely in this group. The comment “Persistent decrease in globulin levels may require the measurement of immunoglobulin” was added to results with globulin levels below the reference range in patients ≥ 18 to < 30 years of age. Clinical information for patients was obtained from electronic notes. If the information in patient electronic notes was incomplete, community physicians were contacted either by email or by telephone, for further clinical details.

Statistical Analysis

After exclusion of duplicates a total of 249 samples were tested for both immunoglobulin and protein electrophoresis, 139 were reflex tested at globulin levels < 16 g/L. One hundred and ten patient samples with globulin levels ≤ 16 g/L and protein electrophoresis and immunoglobulin requested through routine clinical care were included in the analysis. Statistical analysis was performed and graphs constructed using GraphPad Prism (UK). All patient data were analysed and patient confidentiality maintained in accordance with NHS data protection policy^{12,13} and the Declaration of Helsinki.

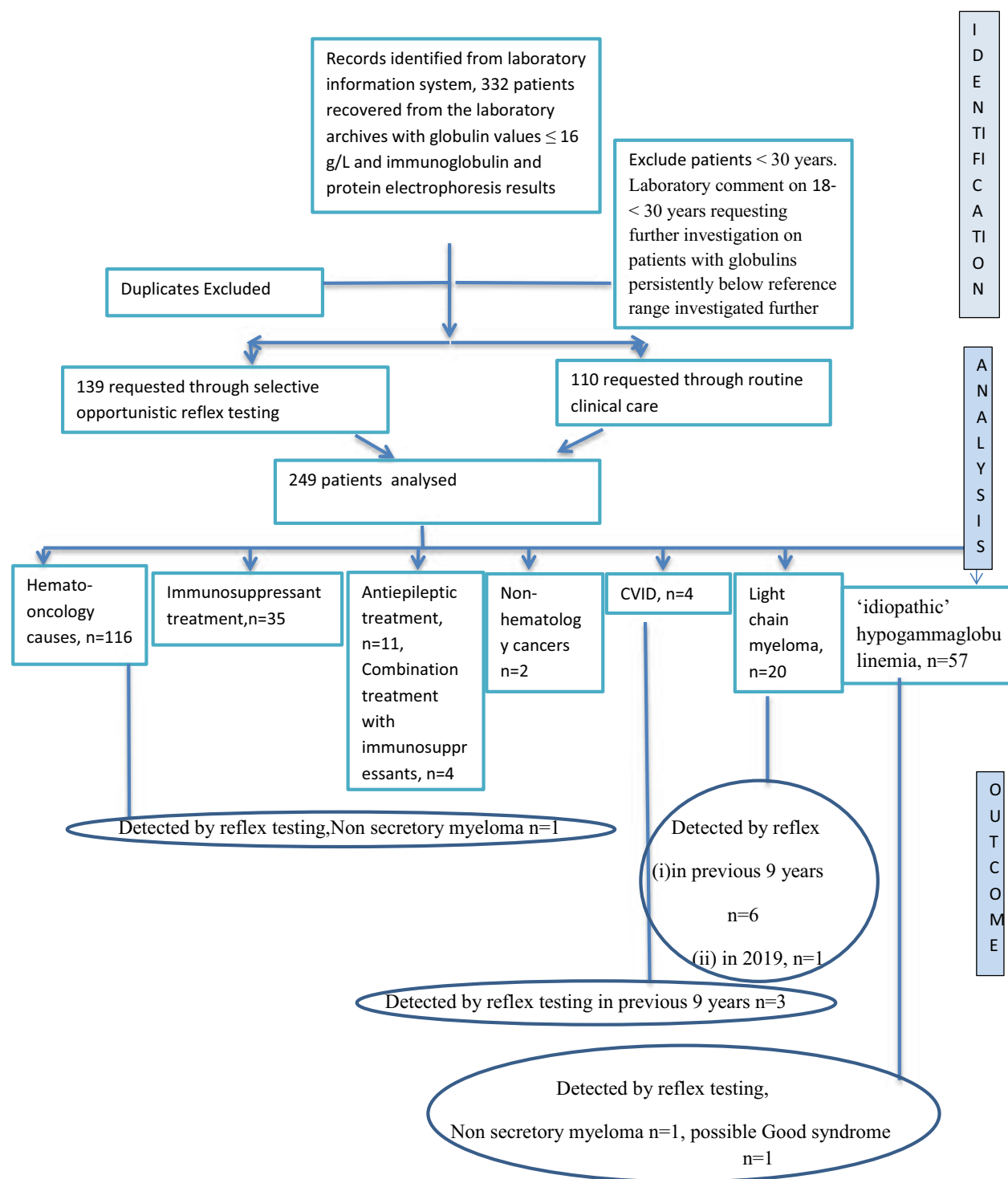


Figure 1 Flow chart of analysis of patient samples.

Results

The mean age of the 249 patients was 70 years (range 33–97), and 50% were females. The IgG concentration according to globulin levels is given in Figure 2. The mean IgG values differed significantly ($p < 0.001$) between each group. The diagram shows a wide distribution of IgG values at each globulin level. It is significant that low IgG levels of < 3 g/L are

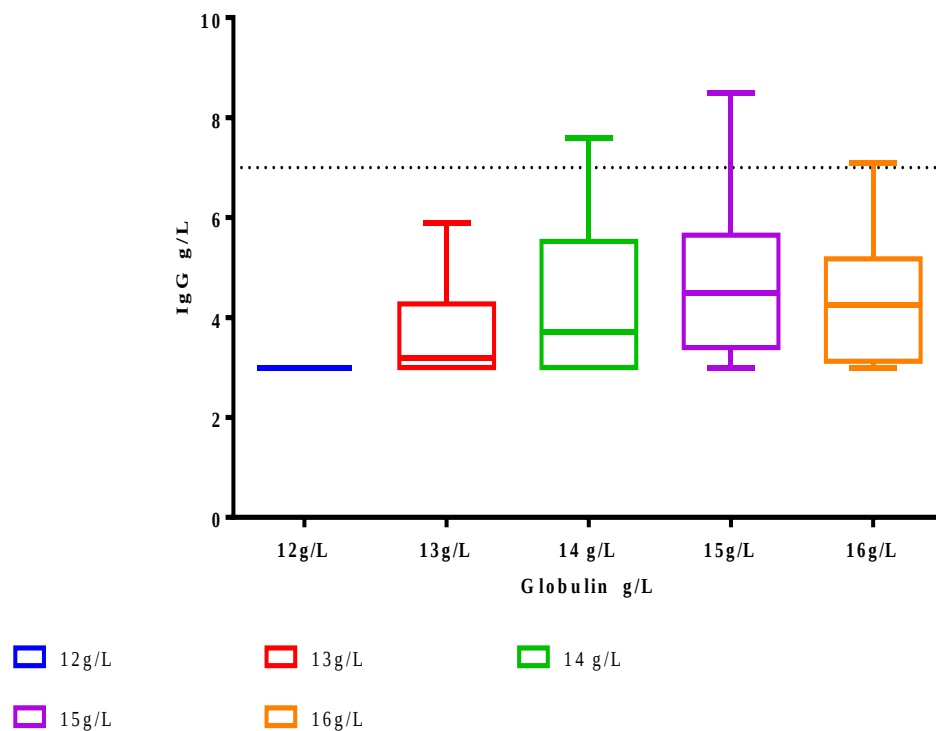


Figure 2 IgG levels at different globulin concentrations. The lower reference range of IgG = 7 g/L is indicated by the horizontal line in the figure. IgG <3 g/L has been converted to 3 g/L in the graph.

found at globulin values of 16 g/L, suggesting that the distribution of decreased IgG levels will extend to globulin levels >16 g/L and that a higher cut-off level may be indicated. A globulin cut-off level of 16 g/L was considered cost effective and practical as it resulted in reflex testing of 2–3 patient sera for protein electrophoresis and immunoglobulin measurements per week. In this group 26% of samples had IgG values ≤ 3 g/L, 49% had IgG values ≤ 4 g/L and 86% had IgG values ≤ 6 g/L, indicating a high positive predictive value for hypogammaglobulinemia. A summary of results according to diagnosis are in Table 1. Follow-up of patients during the year following the first diagnostic sample identified 15/249 patients (globulin values 14–16 g/L) who died (causes varied from co-existing morbidities, $n = 7$; light chain myeloma $n = 3$; hemato-oncology, $n = 5$) during the year of the study.

Patient Subgroup Clinical Details

Hematology Patients

The specialty with highest number samples (116/249, 46.5%) with low globulins was hematology (Figure 3). The diagnostic groups were chronic lymphocytic leukemia (CLL), multiple myeloma, follicular lymphoma, non-Hodgkin lymphoma (NHL), mantle cell lymphoma, marginal zone lymphoma, T-cell lymphoma, Waldenström's macroglobulinemia (WM), IgD myeloma, acute myeloid leukemia (AML), polycythemia rubra vera, acute lymphoid leukemia (ALL) and chronic myeloid leukemia (CML) (Figure 4).

Thirty-six percent of the patients were reflex tested for immunoglobulins and protein electrophoresis based on low globulin values. In these patients with SID those who showed an increased predisposition to infection were treated either with prophylactic antibody or intravenous immunoglobulin. Twelve percent of NHL patients, 20% of CLL patients, 11% of lymphoma patients, and 1 of 3 patients with WM were under prophylactic treatment for SID.

Reflex testing (globulin = 14 g/L) showed a minor paraprotein band (<3 g/L) in a patient who presented with nausea and weight loss. Bone marrow showed extensive plasma cell infiltration and review showed lytic bone lesions. The patient's serum free light chains were kappa = 5.52 mg/L (reference range (RR) 3.3–19.4), lambda = 3.74 mg/L (RR 5.7–26.3) and immunoglobulins IgG 3.1 g/L (RR 7–16), IgA 0.19 g/L (RR 0.7–4.0) and IgM 0.1 g/L (RR 0.4–2.3). The

Table 1 Diagnosis of Patients with Globulin Levels ≤ 16 g/L

	n	IgG Range at Globulin Levels ≤ 16 g/L (RR 7–16 g/L)	Age Years (Range)	Female %	Reflex Tested During Jan 2019–Dec 2019%	Numbers Detected by Reflex Testing Compared to Patients with Hematology Malignancies	% Treated with Prophylactic Antibiotics or Immunoglobulin Replacement	% Referred for Specialist Immunology and Hematology	Numbers Referred for Specialist Immunologist/Hematology Opinion Compared with Patients with Hematology Malignancies
Hematology	116	<3–7 g/L; median: 3.95	72 (34–86)	47	36		12.9%	100%	
Immunosuppressant treatment	35	<3–7.3; median: 4.9	72 (36–88)	61	89	$p < 0.001$	0%	5.7%	$p < 0.001$
Antiepileptic treatment	11	<3–5.0; median: 4.5	60 (33–83)	70	80	$p < 0.001$	0%	0%	$p < 0.001$
Combination treatment with antiepileptics and immunosuppressants	4	<3–5.5; median: 5	72 (57–82)	50	75	*			
Non-hematology malignancy (breast and renal malignancy)	2	<3–3.1; median: 3.1	82, 87	50	50	*			
Light chain myeloma	20	<3–6.7; median: 4.65	70 (48–87)	60	15	$p < 0.001^{**}$	5%	100%	
CVID	4	<3–4; median: 3.7	52 (38–61)	75	100	*			
Patients with non-related co-morbidities	57	<3–8.5; median: 5.5	67 (33–97)	40	82	$p < 0.001$	0%	19.3%	$p < 0.001$

Notes: *Patient numbers < 10 ; **Patients under the care of hematology specialists.

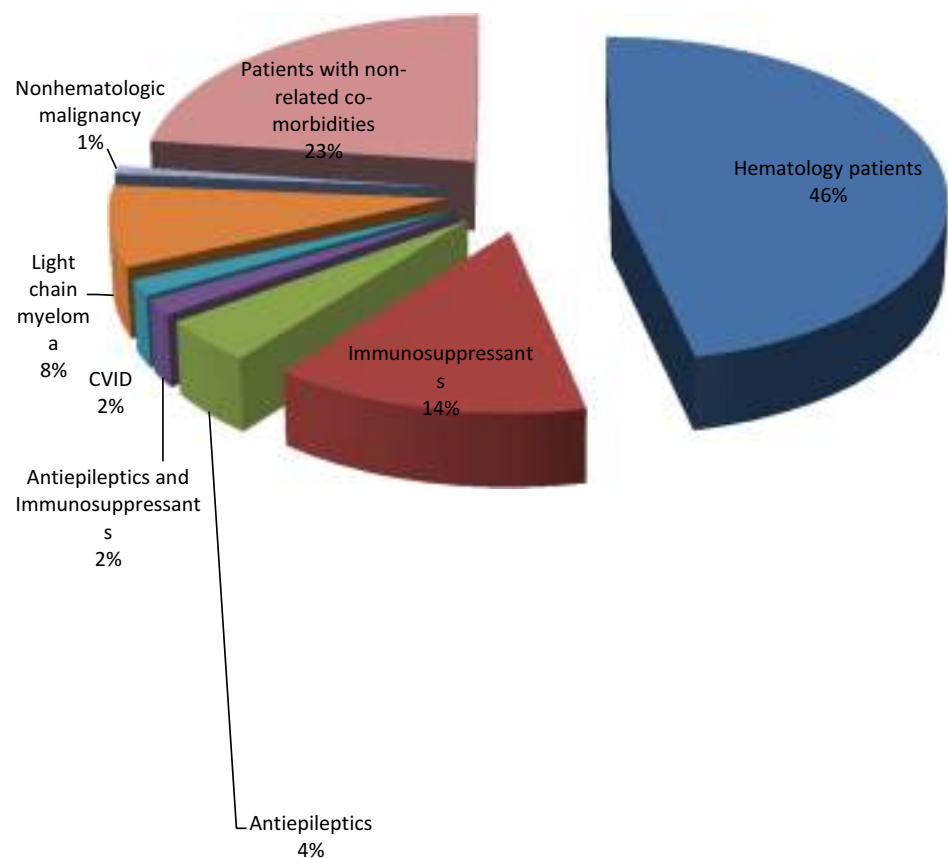


Figure 3 Diagnosis of patients with globulin levels ≤ 16 g/L.

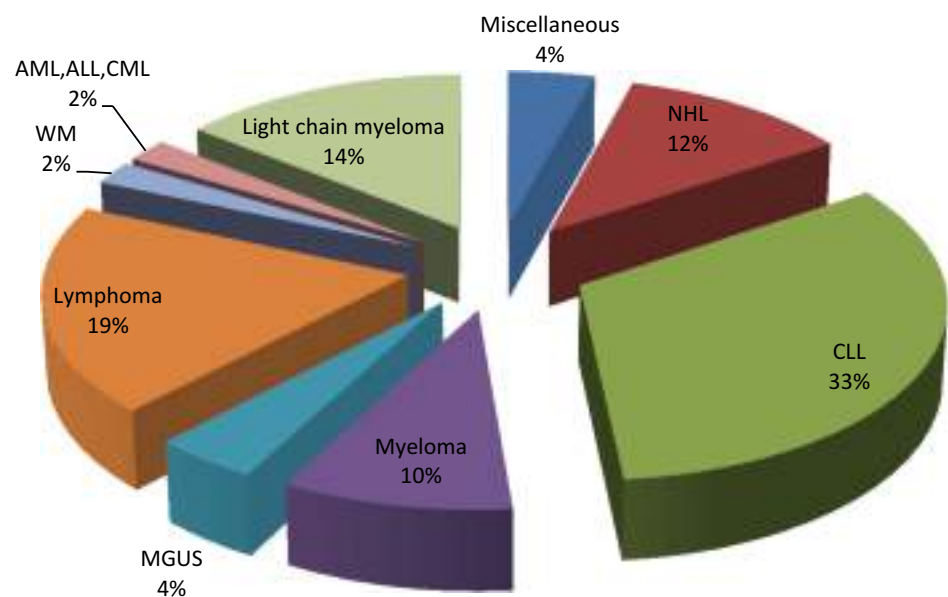


Figure 4 Hematology subgroups.

patient was diagnosed with non-secretory myeloma. In this patient (1/139) screening identified significant disease and the finding of non-secretory myeloma was made following investigation prompted by the detection of immunoparesis.

Immunosuppressant Treatment

Clinical details on patients treated with immunosuppressants were ulcerative colitis, vasculitis, polymyalgia rheumatica, nephrotic syndrome, asthma, giant cell arteritis, rheumatoid arthritis, renal transplant and Crohn's disease. Of the 35 patients on immunosuppressant treatment, 16 were treated with glucocorticoids, 13 were treated with both corticosteroid and immunosuppressants (methotrexate, azathioprine, sulfasalazine, tacrolimus), five were treated with immunosuppressants only and a single patient was on clozapine. Low immunoglobulins were detected by reflex testing in 89% of patients.

Of the 16 patients on glucocorticoid treatment two were under observation for increased infections and possible prophylactic treatment.

Antiepileptic Drugs

Immunoglobulin levels below the reference range were detected in 11 patients treated with antiepileptic drugs. Antiepileptic drugs associated with a reduction in immunoglobulin levels were carbamazepine, phenytoin, gabapentin, lamotrigine, levetiracetam and pregabalin. Four patients were on combined treatment with both antiepileptic drugs and immunosuppressants. Decreased immunoglobulins were detected by reflex testing in 80% and 75% of patients who were on antiepileptic drugs or combined treatment. A single patient on combined treatment with a diagnosis of multiple sclerosis was under the observational care of a specialist in immunology following the report of SID.

Unlike SID linked to hematological malignancies, the approach described here highlights the challenges in identifying and assessing patients with SID secondary to iatrogenic causes and associated with other co-morbidities. In the study cohort, the number of immune deficiency patients identified by automated reflex testing was increased in the latter groups when compared with immunosuppressed patients with hematological malignancies (Table 1, $p < 0.001$). In addition patients were less likely to be referred for immunology/hematology review and further treatment ($p < 0.001$).

Oncology Patients Undergoing Treatment

Low immunoglobulins were detected in two patients with breast or renal cancer and on treatment. Of these one patient was detected with low immunoglobulins by reflex testing with an IgG = 3.1 g/L. Neither patient was under investigation for SID.

Primary Immune Deficiency

Four patients with CVID were seen during the study, three of whom were detected in the previous 9 years by reflex testing and the fourth patient by routine diagnostic request. All three patients were diagnosed by reflex testing prior (globulin levels < 16 g/L) to the study, within the same screening program, and were included in the study as they presented with persistent total globulin values ≤ 16 g/L in 2019. Review by a specialist in immunology and initiation of treatment followed the detection of immunoparesis by reflex testing. Delay in recognition of the underlying diagnosis can result in recurrent infections and irreversible end organ damage such as bronchiectasis. The impact of screening at low globulin levels for the diagnosis of clinically significant disease and the effect of diagnostic delay is shown in example 1 (Supplementary Materials).

Abnormal Serum Free Light Chains

Of the 20 patients with abnormal serum free light chains six were reported by reflex testing, in the 9 years prior to the start of the study (by the same screening program, globulin levels < 16 g/L), four with light chain monoclonal gammopathy of undetermined significance (MGUS) and two with light chain myeloma. All six patients were reviewed by hematologists following the detection of immunoparesis and abnormal serum free light chains. Treatment was commenced on the two patients diagnosed with light chain myeloma. Of the 20 patients 1/20 was treated with prophylactic antibody to prevent infection. Two patients with light chain myeloma were detected following routine requests for immunoglobulin and protein electrophoresis during the study period. A single patient was identified by reflex testing (globulin = 14 g/L) with abnormal serum free light chains kappa = 11.62 mg/L (RR 3.3–19.4), lambda =

250.14 mg/L (RR 5.7–26.3), during the course of the study, a detection rate of 0.7% (1/139) for patients with abnormal serum free light chains. The patient presented with severe back pain treated with morphine. The patient died prior to hematology follow-up. Patient review shows that early diagnosis of myeloma patients can alter the performance status of the patient and define fit versus frail patients and consequent selection for treatment.¹⁴ A further case study (Example 2 is included in [Supplementary Materials](#)) shows the impact of diagnostic delay on treatment. In this patient, there was a delay in diagnosis of light chain myeloma and the patient was found to have vertebral lesions, which can cause severe morbidity.

Patients with Low Globulin Values of Unknown Cause: “Idiopathic” Hypogammaglobulinemia

The study found unsuspected antibody deficiency in 57 (57/249 = 22.9%) patients with other co-morbidities (Figure 5). Low globulins were transient in 11 patients. Eleven patients (11/57, 19%) were referred by community physicians for further review by either hematology or immunology specialist medical practitioners; review of patient notes suggest that myelodysplastic syndrome was suspected in one of these patients and advice was to keep up to date with vaccines in a second patient. A further patient presented with persistently low immunoglobulins from 2014, with immunoglobulin levels: IgG = 3.9 g/L, IgA = 0.43 g/L, IgM = 0.37 g/L. The patient had a past history of heart failure, chronic kidney disease, hypertension and osteoarthritis. The patient treatment included thyroxine, amlodipine, fluoxetine, warfarin, bisoprolol and simvastatin. The patient did not present with clinical symptoms of increased infection or night sweats and was assigned to monitoring.

Eight patients (8/57, 14%) were tested for specific antibody response to vaccines, and 5/57 (9%) showed a decreased antibody response to tetanus, hemophilus and pneumococcus challenge. Immunology advice was requested for 1/5 patients, with subnormal vaccine response and IgG <3 g/L. Advice from specialist immunologist was to keep up to date with vaccinations, and for further specialist immunology opinion if there was an increase in rate of infections. A patient with IgG = 4.3 g/L, was not followed up further. Three further patients with IgG = 4.0 g/L, IgG = 4.8 g/L and IgG = 4.7 g/L with several co-morbidities (depression, frailty, Alzheimer’s disease) were assigned to monitoring should symptoms develop.

The remaining 27 patients presented with several co-morbidities (myocardial infarction, hemochromatosis, Parkinson’s disease). This cohort included the following: two patients who presented with recurrent UTI, with an IgG

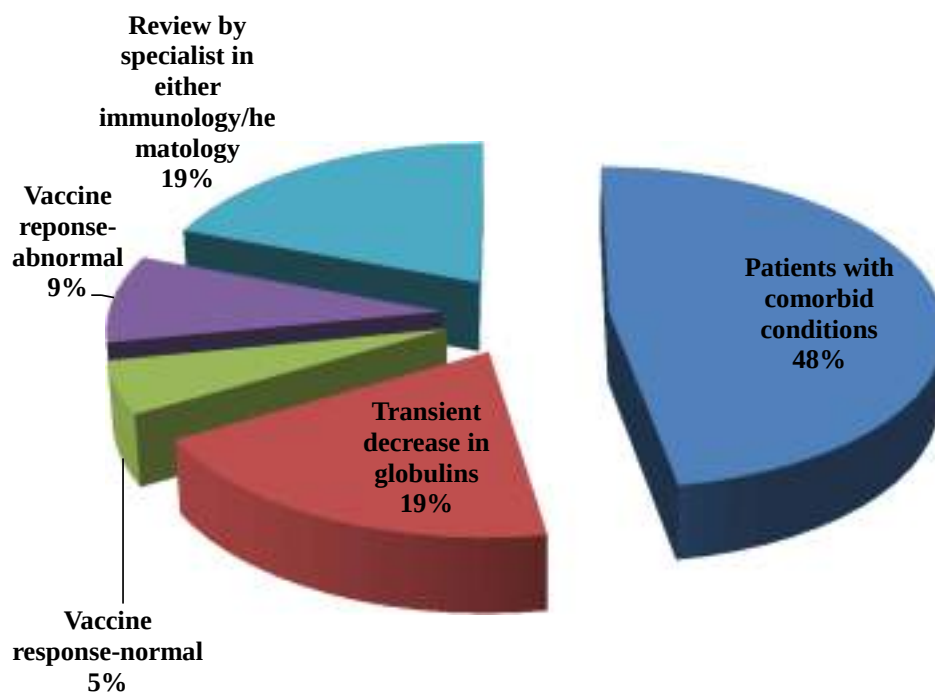


Figure 5 Follow-up details on patients with globulin ≤ 16 g/L, idiopathic hypogammaglobulinemia.

5.4 g/L and antibody response to vaccines within reference range, and IgG 4.8 g/L who were not examined further. A further patient with a prior history of testicular and colorectal cancer and low calculated globulin was found to have low immunoglobulins by reflex testing in 2019 (IgG = 3.3 g/L, IgA = 0.77 g/L, IgM = 0.43 g/L). The patient's immunoglobulins remained below reference range in 2021, and serum free light chains and antibody response to tetanus, hemophilus and pneumococcus challenge were within reference range. The incidence of malignancy has been stated as increased in CVID, regular follow-up may be advantageous in this patient should new problems arise.¹⁵ It is likely that in patients who during the period of observation were asymptomatic, potential interventions such as antibiotic prophylaxis and treatment and vaccination were not indicated. Treatment may depend on progressive decline in antibody levels and the development of symptoms.¹⁶

However, examples 3 and 4 in [Supplementary Materials](#) demonstrate the complexity of clinical presentation among the patients classified as idiopathic hypogammaglobulinemia and are examples of patients who would have benefited from earlier as well as more extensive investigation. The examples include a further 1/139 patients in the detection of new, previously unsuspected non-secretory MM during the course of the study.

The study shows the difficulty associated with the diagnosis of “idiopathic hypogammaglobulinemia”. This cohort of patients includes those with transient hypogammaglobulinemia as well as patients with comorbidities who were placed on surveillance should symptoms develop and treatment necessitated. Nineteen of the 57 patients (33%) were further investigated either by referral to hematologists/immunologists or by antibody response to vaccines. Patients were placed under observation for further review should symptoms develop. In these patients presentation is less defined and it is a challenge to determine whether or not to treat. Of the 57 patients two more patients were identified, one of whom with a possible diagnosis of Good syndrome who presented with sepsis would have benefitted from further investigation and treatment. The second patient underwent further investigation for anemia and gastroenterology review prior to diagnosis of non-secretory myeloma. Additional investigation was prompted by this study, following a detection of immunoparesis in 2019 by reflex testing. A summary of diagnostic conclusions in the distinct groups is given in [Table 2](#).

Table 2 Summary of Diagnostic Outcomes

Diagnosis	Globulin Level at Diagnosis	Outcome
Light chain myeloma	14 g/L	Identified during the course of the study, presented with severe back pain on morphine treatment. Died prior to initiation of treatment for myeloma.
Light chain myeloma	<16 g/L	Identified in the previous 9 years by reflex testing. All six patients were reviewed by hematology specialists; four with light chain MGUS were placed under observation, and treatment initiated for two patients with light chain myeloma.
Non-secretory myeloma	14 g/L	Two patients identified by reflex testing during the course of the study, treatment was commenced for non-secretory myeloma by hematology specialists.
Possible Good syndrome	15 g/L	Frail patient assigned to conservative treatment.
CVID	<16 g/L	Three patients identified with globulin < 16 g/L in 2019, were identified in the previous 9 years by reflex testing. Treatment was initiated by immunology specialists following diagnosis of CVID.
“Idiopathic hypogammaglobulinemia”	<16 g/L	This cohort included transient hypogammaglobulinemia and those with co-existing morbidities. In this group 33% were further investigated either by specialists in immunology or hematology, or by antibody response to vaccines. The group were either assigned to monitoring should symptoms develop or advised on vaccination schedules or antibiotic treatment. Patients who had a delayed diagnosis of non-secretory myeloma, possible Good syndrome or possible CVID were allocated to this group prior to further investigation and diagnostic testing.
SID	<16 g/L	The group included patients with immunoparesis secondary to hematological malignancies or immunosuppressant or antiepileptic drug therapy. The latter two groups were more likely to be detected by reflex testing and less prone to be reviewed for SID.

Discussion

Calculated globulin values have previously been suggested as a useful screening tool for detection of antibody deficiency or small paraprotein bands with immunoparesis,¹⁷ however real life evidence supporting this practice is lacking. In WRH protein electrophoresis and measurement of immunoglobulin levels at globulin levels below the reference range were carried out both as a reflex test and as requests through routine clinical care. Both approaches were used to complement each other in the diagnosis of immune deficiency and MM with immunoparesis. The main findings of this study are that both MM with immunoparesis and primary immune deficiency can be detected by this approach. This study further demonstrates that the program detected previously unsuspected secondary antibody deficiencies.

The current cut-off for screening is globulin values ≤ 16 g/L. The current study suggests low values of IgG < 3 g/L are found at globulin value of 16 g/L, suggesting a higher cut-off value may detect low values of IgG. The value of 16 g/L was considered a practical solution in that 2–3 patient samples were detected per week and 86% of the IgG values detected were below 6 g/L. In WRH selective reflex testing of samples sent to the laboratory in conjunction with requests for investigation of low globulin levels through routine clinical care was considered a practical and cost-effective investigative approach. One previous study suggests that the use of the first percentile of the globulin fraction improved the detection of hypogammaglobulinemia.¹⁷

Screening identified approximately 2.2% of the 139 patients screened during 2019 with previously undiagnosed MM. Myeloma is characterized by bone lesions, anemia, renal impairment and immune deficiency. The patients may require prophylaxis and supportive treatment for osteolytic pain, anemia, renal insufficiency, peripheral neuropathy and thromboembolic events. Delay in diagnosis may lead to impairment in quality of life, life expectancy as well as increased health-care costs.¹⁴

The study identified three patients with CVID and six patients with light chain myeloma detected by reflex testing, within the same screening program, prior to the start of the study period. This data suggests that both CVID and MM are detected by reflex testing, and that clinically significant disease can be detected by the screening criteria. The patients presented with globulin levels ≤ 16 g/L and were detected during the study period by the study protocol. It is possible that additional patients diagnosed with CVID or light chain myeloma, by the WRH screening program, prior to the start of the study were under treatment and may no longer present with globulin levels ≤ 16 g/L. Previous reviews using screening based on globulin cut-off levels, serum electrophoresis and immunofixation detected small paraproteins associated with immunoparesis in 1.2% of samples.⁷

CVID is one of the common primary immune deficiency disorders encountered in clinical practice. CVID can present with chronic infections of the sinopulmonary tract, however clinical manifestations can be broad and include autoimmunity, splenomegaly, lymphadenopathy as well as an increased incidence of cancers. Genomic studies have revealed an increasing number of genetic causes of the CVID phenotype.¹⁸ In studies with CVID patients a mean diagnostic delay of 7.46 years has been observed. As the presentation of CVID may include non-infectious autoimmune or inflammatory conditions diagnostic delay can result in inappropriate treatment.¹⁹ Identification and investigation of patients with low globulin values has the potential to decrease the delay in making the diagnosis.

Secondary immune deficiency caused by hematological malignancies was identified in 46.6% of patients, a significant proportion of the patients were identified by routine clinical requests and a noteworthy number of patients were under treatment for SID. In contrast, a substantial number of patients with SID as a result of antiepileptic or immunosuppressant therapy or a combination of both were identified by reflex testing and an insignificant number of patients were under treatment for SID. Neither of the two patients with non-hematological malignancy and low immunoglobulins were investigated further. With the growth of new treatments for non-hematological oncology patients monitoring of immunoglobulins for SID with a possibility of non-neutropenic sepsis may be indicated. The current study excluded non-hematology oncology patients from reflex testing for protein electrophoresis and immunoglobulins; inclusion of these patients may detect patients at increased risk of hypogammaglobulinemia. It remains to be seen if patients with hypogammaglobulinemia at increased risk of malignancy can be identified by this approach.

Clinical routine requests for immunoglobulins and protein electrophoresis were less likely if the patients were treated with immunosuppressive drugs, antiepileptic drug therapy or clozapine. Patients on clozapine can display clinical

patterns resembling CVID.²⁰ In patients with iatrogenic SID, investigation to assess response to vaccination and a trial of prophylactic antibiotic treatment may be indicated. This study suggests limitations in the current response to iatrogenic SID. Further studies are required to define adequacy and type of treatment needed in these patients.²¹

A further 22.9% with several co-morbidities and non-specific symptoms were identified with hypogammaglobulinemia and a significant number were detected by reflex testing. Transient hypogammaglobulinemia was identified in 19% of this cohort and further advice for a second 19% of the cohort from either hematologists/immunologists identified patients with myelodysplasia or who required up to date vaccination. Of the 9% of the cohort who showed a subnormal response to vaccines, immunology advice was requested for a single patient. There may be instances where patients are assigned to monitoring, however if patients develop symptoms, treatment (e.g. antibiotic administration) may be altered in light of the observed hypogammaglobulinemia. Review of the remaining patients in this cohort identified patients with decreased immunoglobulins (possible Good syndrome, a second with malignancy and a further patient with non-secretory myeloma) who would have benefitted from further investigation.

A number of approaches can be taken to raise the awareness of community physicians to immune deficiency and MM as part of the differential diagnosis of hypogammaglobulinemia as early patient encounters will be with non-specialist community physicians. Coded comments attached to the results can direct the physician to appropriate testing. Another approach is to use direct comments in results to increase awareness of immune deficiency and draw the clinician's attention to documents (Jeffrey Modell Foundation (JMF) "10 Warning Signs of Immune deficiency" which are widely available; <http://www.info4pi.org/library/educational-materials/10-warning-signs>)^{22,23} and describe symptoms associated with immune deficiency. Additionally, the UK myeloma forum has guidelines for the diagnosis and treatment of myeloma (<https://www.ukmf.org.uk/guidelines-page/bshukmf-guidelines/>).

In WRH, the cost of screening using protein electrophoresis and immunoglobulins is £5:00/test at 2–3 patient requests per week. The study suggests a detection rate of approximately 2.2% for MM during 2019. A review of patients in the study suggests that both CVID and light chain MM were identified by reflex testing in the years prior to 2019. In addition, patients with SID who required further investigation were identified. The economic impact of the screening method has to be balanced by the possibility of decrease in time delay in diagnosis and detection of disease before the onset of end-organ damage which can affect long-term prognosis and the quality of life in patients with immune deficiency and multiple myeloma.^{14,24} A strong case for detecting antibody deficiency using discretionary laboratory testing at calculated globulin levels below the reference range has been suggested,⁷ though there are few studies in routine laboratories to investigate its effectiveness. Previous reviews have emphasized the necessity of early diagnosis to prevent complications for both immune deficiency²⁵ and MM.²⁶ Cost-analysis of renal dialysis, should end-organ damage occur in MM, estimates an annual cost of approximately £30,000 per patient for hospital-based hemodialysis in the UK.²⁷ In the USA pneumonia is a major cause of mortality and morbidity and the immunocompromised population can experience a disproportionate burden of pneumonia-related hospitalization. In the USA the annual charge for pneumonia-related hospitalization is over \$84 billion.²⁸ On average the cost of reflex testing for immunoglobulins and protein electrophoresis at globulin levels was approximately $136 \times 5 = £680$ per year. The burden on finite health-care budgets was an important consideration in devising the screening program. The annual detection rate of 2.2%, with an estimated cost of £227 (680/3)/patient detected, suggests an economic benefit to the health-care system.

This is a single center study and bias may have been introduced due to referral characteristics. A limitation of this study is that it evaluated patients ≥ 30 years of age. Potential further studies would be to assess the value of investigating globulin values ≤ 16 g/L at < 30 years. In the current economic environment the challenge is to appraise an acceptable detection rate without a substantial increase in service cost. The practice at WRH is to use both discretionary screening tests as well as routine requests for immunoglobulins and protein electrophoresis for the diagnosis of both immune deficiency and multiple myeloma at globulin values below the reference range. Screening programs differ in their protocol and the procedure at WRH can be compared with other programs to assist future decisions on the best method to aid in the diagnosis of both MM and immunodeficiency by following globulin levels below the reference range. To our knowledge, this is the first study to include the two different diagnoses within the same program.

Conclusion

The current globulin cut-off value of ≤ 16 g/L identified both immune deficiency and MM patients. The results show that this approach can detect patients with immunoparesis, thus contributing to inappropriate investigation and delay in treatment. The study identified patients with secondary immune deficiency as well as idiopathic hypogammaglobulinemia. The latter patients were a heterogeneous group who needed close clinical and laboratory assessment for the development of optimal treatment strategies.

Data Sharing Statement

The data are stored in the Laboratory Quality Management System of The Worcester Royal Hospital, and are available following anonymization of patient identifiable information. The full data set can be requested from the corresponding author upon approval of the paper proposal using the data.

Ethics Approval and Consent to Participate

The study was an audit carried out according to the Declaration of Helsinki and the NHS Data Protection Act. Approval for the study was granted by the Research and Ethics Committee of the Worcester Acute Hospitals NHS Trust as per NHS Data Protection Act. Informed verbal consent was obtained from patients and approved by the Research and Ethics Committee of the Worcester Acute Hospitals NHS Trust.

Consent for Publication

The study is a service audit (carried out as a cross-sectional observational study, according to STROBE guidelines). Informed verbal consent was obtained from patients and approved by the Research and Ethics Committee of the Worcester Acute Hospitals NHS Trust.

Author Contributions

I am the sole author of the manuscript and made a significant contribution to the work reported, in the conception, study design, execution, acquisition of data, analysis and interpretation; took part in drafting, revising and critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The author reports no competing interests in this work.

References

1. Zhang J, Sun W, Huang Z, et al. Light chain multiple myeloma, clinic features, responses to therapy and survival in a long-term study. *World J Surg Oncol*. 2014;12:234. doi:10.1186/1477-7819-12-234
2. Dupuis MM, Tuchman SA. Non-secretory multiple myeloma: from biology to clinical management. *Once Targets There*. 2016;9:7583–7590. doi:10.2147/OTT.S122241
3. Dilley M, Wangberg H, Noone J, Geng B. Primary immunodeficiency diseases treated with immunoglobulin and associated comorbidities. *Allergy Asthma Proc*. 2021;42(1):78–86. doi:10.2500/aap.2021.42.200113
4. Cunningham-Rundles C. Common variable immune deficiency: dissection of the variable. *Immunol Rev*. 2019;287(1):145–161. doi:10.1111/imr.12728
5. Patel SY, Carbone J, Jolles S. The expanding field of secondary antibody deficiency: causes, diagnosis, and management. *Front Immunol*. 2019;10:33. doi:10.3389/fimmu.2019.00033
6. Greenberger PA, Walker CL, Fitzsimons TE, Roberts M. Hypogammaglobulinemia associated with cytomegalovirus pneumonia. *J Infect Dis*. 1991;163(3):631–633. doi:10.1093/infdis/163.3.631
7. Holding S, Jolles S. Current screening approaches for antibody deficiency. *Curr Opin Allergy Clin Immunol*. 2015;15(6):547–555. doi:10.1097/ACI.0000000000000222
8. Jolles S, Borrell R, Zouwail S, et al. Calculated globulin (CG) as a screening test for antibody deficiency. *Clin Exp Immunol*. 2014;177(3):671–678. doi:10.1111/cei.12369
9. Hoo T, Lim EM, John M, D'Orsogna L, McLean-Tooke A. Calculated globulin as a screening tool for hypogammaglobulinaemia or paraproteins in hospitalized patients. *Ann Clin Biochem*. 2021;58(3):236–243. doi:10.1177/0004563221989737
10. Shields AM, Burns SO, Savic S, Richter AG; UK PIN COVID-19 Consortium. COVID-19 in patients with primary and secondary immunodeficiency: the United Kingdom experience. *J Allergy Clin Immunol*. 2021;147(3):870–875. doi:10.1016/j.jaci.2020.12.620

11. Adams RA, Smith L, Pickering PE. The incidence of monoclonal proteins during 7 years of screening in a district general hospital. *Immunology*. 1984;51(3):451–454.
12. Twycross A, Shorten A. Service evaluation, audit and research: what is the difference? *Evid Based Nurs*. 2014;17(3):65–66. doi:10.1136/eb-2014-101871
13. NHS England and NHS Improvement. Data Protection Policy. Available from: <https://www.england.nhs.uk/wp-content/uploads/2019/10/data-protection-policy-v5.1.pdf>. Accessed May 24, 2023.
14. Terpos E, Kleber M, Engelhardt M, et al.; European Myeloma Network. European Myeloma Network guidelines for the management of multiple myeloma-related complications. *Haematologica*. 2015;100(10):1254–1266. doi:10.3324/haematol.2014.117176
15. Cunningham-Rundles C. How I treat common variable immune deficiency. *Blood*. 2010;116(1):7–15. doi:10.1182/blood-2010-01-254417
16. Ameratunga R, Ahn Y, Steele R, Woon ST. The natural history of untreated primary hypogammaglobulinemia in adults: implications for the diagnosis and treatment of Common Variable Immunodeficiency Disorders (CVID). *Front Immunol*. 2019;10:1541. doi:10.3389/fimmu.2019.01541
17. Holding S, Khan S, Sewell WA, Jolles S, Dore PC. Using calculated globulin fraction to reduce diagnostic delay in primary and secondary hypogammaglobulinaemias: results of a demonstration project. *Ann Clin Biochem*. 2015;52(Pt 3):319–326. doi:10.1177/0004563214545791
18. Maffucci P, Filion CA, Boisson B, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front Immunol*. 2016;7:220. doi:10.3389/fimmu.2016.00220
19. Cunningham-Rundles C. Common variable immune deficiency: case studies. *Blood*. 2019;134(21):1787–1795. doi:10.1182/blood.2019002062
20. Ponsford MJ, Steven R, Bramhall K, et al. Clinical and laboratory characteristics of clozapine-treated patients with schizophrenia referred to a national immunodeficiency clinic reveals a B-cell signature resembling common variable immunodeficiency (CVID). *J Clin Pathol*. 2020;73(9):587–592. doi:10.1136/jclinpath-2019-206235
21. Jolles S, Chapel H, Litzman J. When to initiate immunoglobulin replacement therapy (IGRT) in antibody deficiency: a practical approach. *Clin Exp Immunol*. 2017;188(3):333–341. doi:10.1111/cei.12915
22. Arkwright PD, Gennery AR. Ten warning signs of primary immunodeficiency: a new paradigm is needed for the 21st century. *Ann N Y Acad Sci*. 2011;1238:7–14. doi:10.1111/j.1749-6632.2011.06206.x
23. Subbarayan A, Colarusso G, Hughes SM, et al. Clinical features that identify children with primary immunodeficiency diseases. *Pediatrics*. 2011;127(5):810–816. doi:10.1542/peds.2010-3680
24. Graziano V, Pecoraro A, Mormile I. Delay in diagnosis affects the clinical outcome in a cohort of CVID patients with marked reduction of IGA serum levels. *Clin Immunol*. 2017;180:1–4. doi:10.1016/j.clim.2017.03.011
25. Sánchez-Ramón S, Bermúdez A, González-Granado LI, Rodríguez-Gallego C, Sastre A, Soler-Palacín P; ID-Signal Onco-Haematology Group. Primary and secondary immunodeficiency diseases in oncohaematology: warning signs diagnosis, and management. *Front Immunol*. 2019;10:586. doi:10.3389/fimmu.2019.00586
26. Rajkumar SV. Evolving diagnostic criteria for multiple myeloma. *Hematol Am Soc Hematol Educ Program*. 2015;2015:272–278. doi:10.1182/asheducation-2015.1.272
27. Roberts G, Holmes J, Williams G, et al. Current costs of dialysis modalities: a comprehensive analysis within the United Kingdom. *Perit Dial Int*. 2022;42(6):578–584. doi:10.1177/08968608211061126
28. Hayes BH, Haberling DL, Kennedy JL, Varma JK, Fry AM, Vora NM. Burden of pneumonia-associated hospitalizations: United States, 2001–2014. *Chest*. 2018;153(2):427–437. doi:10.1016/j.chest.2017.09.041
29. Kelleher P, Misbah SA. What is Good's syndrome? Immunological abnormalities in patients with thymoma. *J Clin Pathol*. 2003;56(1):12–16. doi:10.1136/jcp.56.1.12

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Platelet Selectin Levels in Patients with Cerebral Venous Sinus Thrombosis: Preliminary Findings

Eswi Zenobia, Nushrotul Lailiyya, Sofiati Dian, Cep Juli, Asep Nugraha Hermawan & Lisda Amalia

To cite this article: Eswi Zenobia, Nushrotul Lailiyya, Sofiati Dian, Cep Juli, Asep Nugraha Hermawan & Lisda Amalia (2023) Platelet Selectin Levels in Patients with Cerebral Venous Sinus Thrombosis: Preliminary Findings, *Journal of Blood Medicine*, , 359-365, DOI: [10.2147/JBM.S405975](https://doi.org/10.2147/JBM.S405975)

To link to this article: <https://doi.org/10.2147/JBM.S405975>



© 2023 Zenobia et al.



Published online: 31 May 2023.



Submit your article to this journal



Article views: 205



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Platelet Selectin Levels in Patients with Cerebral Venous Sinus Thrombosis: Preliminary Findings

Eswi Zenobia, Nushrotul Lailiyya, Sofiati Dian , Cep Juli, Asep Nugraha Hermawan, Lisda Amalia 

Neurology Department, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Correspondence: Lisda Amalia, Neurology Department, Faculty of Medicine, Universitas Padjadjaran, Jl. Eykman 38 Bandung, Bandung, 40161, Indonesia, Email dr.lisda@gmail.com

Background: Cerebral Venous Sinus Thrombosis (CVST) is a cerebrovascular disease with an estimated annual incidence of 3–4 cases per 1 million population with an 8% mortality rate caused by hypercoagulable conditions and hyper aggregation and also Platelet Selectin (P-Selectin) as one of coagulation biomarker for both of them. This study aimed to describe the levels of P-selectin in CVST patients at RSHS Bandung.

Objective: This study aimed to describe the levels of P-selectin in CVST patients at RSHS Bandung.

Methods: This is a descriptive observational study on patients ≥ 18 years old diagnosed with CVST at the Neurology outpatient polyclinic of RSUP Dr. Hasan Sadikin Bandung for March-May 2022. All samples that meet the inclusion criteria will be included as research subjects.

Results: There were 55 research subjects with a median age of 48 (range 22–69 years), the majority were women (80%), the most complaints were headaches (92.7%), the majority onset was chronic (96.4%) with a length of treatment ≥ 12 months (61.8%). P-selectin levels were found to increase in the group of subjects with subacute onset (mean 5.20 ± 2.977), infectious etiology (mean 5.26 ± 3.561), duration of treatment < 3 Months (mean 3.79 ± 3.065), history of hyper aggregation (mean 3.89 ± 2.805), hypercoagulation (mean 3.50 ± 2.719), increased D-dimer (mean 3.93 ± 2.710), normal fibrinogen (mean 3.38 ± 2.693), and in the group with multiple affected sinuses (mean 6.08 ± 2.681).

Conclusion: P-selectin could be a diagnostic marker for hyper aggregation and hypercoagulable state in patients with CVST, but it still needs further research to prove it.

Keywords: Cerebral Venous Sinus Thrombosis, P-selectin, platelet hyperaggregation, hypercoagulation

Introduction

Cerebral Venous Sinus Thrombosis (CVST) is a cerebrovascular disease in the form of thrombus occlusion in the venous and cerebral sinuses.¹ The incidence rate per year is estimated at ^{2,3} cases per 1 million population with a mortality rate of 8%.^{1,2,4} Patients with CVST are more numerous, especially in young adults than the elderly, with a median age of 45.^{1–4}

The cause of 22–25% of thrombosis in CVST is hyper platelet aggregation caused by dysfunction and disruption of the endothelial structure due to inflammation which triggers platelet activation, which begins with platelet adhesion and continues with the process of secreting platelet granules and forming platelet aggregation.^{4–6} P-selectin levels can assess impaired function of platelet adhesion and secretion.^{7–9}

P-selectin is a transmembrane glycoprotein identified using a monoclonal antibody specific for platelets that activates thrombin.^{7,10,11} P-selectin is a molecule that plays a role in platelet adhesion.^{12,13} Activated platelets will undergo granule fusion with the endothelial plasma membrane, causing exposure of P-selectin to the surface of activated platelets. P-selectin causes adhesion and granule secretion, which in turn causes platelet aggregation.^{12,13} Disruption of platelet function can be predicted by examining P-selectin, which is the result of endothelial and platelet activation and can describe the initial process of thrombosis.^{7,10,11,13} P-selectin is said to have a higher specificity, namely 87%, when compared with D-dimer and fibrinogen because it specifically describes the hypercoagulable process that causes

thrombus formation in veins.¹⁴ P-selectin may be used as a diagnostic tool with D-dimer and fibrinogen examination to diagnose CVST so that patients can be handled more quickly and precisely.

Methods

Sample Size

Sampling was carried out using consecutive sampling from all patients who were ≥ 18 years old and diagnosed with Cerebral Venous Sinus Thrombosis by a neurologist in the cerebrovascular subdivision based on radiological findings (Digital Subtraction Angiography or Magnetic Resonance Venography) and laboratory findings (D-dimer level and coagulation profile) at the Outpatient Neurology Polyclinic at RSUP Dr. Hasan Sadikin Bandung in March-May 2022. There is a lack of examination materials or an insufficient number of samples, patients with hypertension, dyslipidemia, smoking, pre-eclampsia, sepsis, Alzheimer's disease, consuming GP IIb-IIIa agonist, clopidogrel, aspirin, heparin, protamine sulfate, aprotinin, heparinase, insulin, cocaine, and nitric oxide as exclusion criteria. Subjects who met the inclusion criteria expressed their willingness to participate in this study by signing informed consent after obtaining an explanation. The ethics committee of Dr. Hasan Sadikin Hospital Bandung received the research ethics approval under the ethical clearance number LB.02.01/X.6.5/119/2022 and complies with the Declaration of Helsinki. Writing informed consent was obtained from the study participants.

Data Collection

Research subjects were selected at the Neurology Outpatient Polyclinic of RSUP Dr. Hasan Sadikin Bandung obtained from patients who are routinely re-controlled at the polyclinic (a chronic case). Research materials were examined at the Clinical Pathology Laboratory of RSUP Dr. Hasan Sadikin Bandung.

P-Selectin Measurement

Blood samples were taken in the morning after the patient had fasted for at least 8 hours. The serum was separated by allowing blood to clot for 30 minutes and then centrifuging 3500 RPM for 20 min. It was stored at -20°C if not directly processed. The Enzyme-linked immune-sorbent assay was done according to the manufacturer's protocol.

Statistical Analysis

This descriptive analysis is used to determine the characteristics of the research subjects who are the research samples. Data analysis to describe the proportion of each variable that will be presented descriptively can be broken down into a descriptive study. Data on a numerical scale, such as the patient's age, are presented with the mean, standard deviation, median, and range. Then for sample characteristic data in the form of categorical data, such as the sex of the patient, coding is given and presented as a frequency and percentage distribution. The data obtained are recorded in a special form and then processed through the SPSS program version 24.0 for Windows.

Results

A total of 55 research subjects met the inclusion criteria and signed informed consent during the study ([Supplementary Material](#)). The mean age was 45.04 ± 12.100 years, and most research subjects were women. The participant's demographic distribution is described in [Table 1](#).

P-Selectin was highest in the subacute onset group (2 days–30 days) with an average of 5.20 ± 2.977 . The highest group for the infectious risk factor, with an average of 5.26 ± 3.561 . Study subjects with treatment duration <3 Months had the highest average P-selectin value of 3.79 ± 3.065 . P-Selectin with high D-dimer had an average of 3.93 ± 2.710 , and normal fibrinogen had the highest standard of 3.38 ± 2.693 . Subjects with a history of hyper aggregation had an average P-selectin level of 3.89 ± 2.805 .

Whereas research subjects with a history of hypercoagulation had an average of 3.50 ± 2.719 . Multiple affected sinuses had the highest average P-selectin of 6.08 ± 2.681 ([Table 2](#)).

Table I Demographic Characteristics of Participants

Variable	N=55
Age	
Mean±Std	45.04±12.100
Median	48.00
Range (min-max)	22.00–69.00
Gender	
Man	11 (20.0%)
Woman	44 (80.0%)
Complaint	
Headache	51 (92.7%)
Hemiparesis	10 (18.2%)
Visual disturbance	4 (7.3%)
Seizure	1 (1.8%)
Dizziness	3 (5.5%)
Onset	
Acute (≤48 hours)	0 (0.0%)
Subacute (48 hours-30 days)	2 (3.6%)
Chronic (>30 days)	53 (96.4%)
Duration of treatment	
<3 months	15 (27.3%)
3–6 months	5 (9.1%)
6–12 months	1 (1.8%)
≥12 months	34 (61.8%)
Diagnostic Imaging	
CT Scan	18 (32.7%)
Magnetic Resonance Venography	28 (50.9%)
Digital Subtraction Angiography	9 (16.4%)
Sinus Involvement	
Transversus Sinus	39 (70.9%)
Sagittal superior Sinus	7 (12.7%)
Sigmoid Sinus	2 (3.6%)
Cavernous Sinus	1 (1.8%)
Multiple Sinus	6 (10.9%)
D-Dimer (N=49)	
Mean±Std	0.85±1.749
Median	0.33
Range (min-max)	0.19–11.39
Fibrinogen (N=36)	
Mean±Std	380.32±108.362
Median	365.50
Range (min-max)	204.00–738.00
Risk Factor	
Autoimmune	7 (12.7%)
Infection	6 (10.9%)
Oral Contraception	13 (23.6%)
Malignancy	3 (5.5%)
Head Injury	4 (7.3%)
Unknown	22 (40.0%)
Platelet Aggregation Test	
Hypoaggregation	9 (16.4%)
Normoaggregation	21 (38.2%)
Hyperaggregation	25 (45.5%)

(Continued)

Table I (Continued).

Variable	N=55
Thromboelastography (N=29)	
Normocoagulation	9(31.0%)
Hypercoagulation	20(69.0%)
P Selectin levels	
Mean±Std	3.09±2.592
Median	2.15
Range (min-max)	0.07–10.05

Table 2 Platelet Selectin Level Based on Characteristics of Research Subjects

Variable	N (%)	P-Selectin Levels Mean±Std
Onset		
Acute	0	-
Subacute	2 (3.6%)	5.20±2.977
Chronic	53 (96.3%)	3.01±2.575
Risk Factor		
Autoimmune	7 (12.7%)	3.86±3.170
Infection	6 (10.9%)	5.26±3.561
Oral Contraception	13 (23.6%)	3.45±2.591
Malignancy	3 (5.4%)	1.68±1.558
Head Injury	4 (7.2%)	0.98±0.724
Unknown	22 (40%)	2.62±2.064
Duration of treatment		
<3 months	15 (27.2%)	3.79±3.065
3–6 months	5 (9%)	2.63±2.986
6–12 months	1 (1.8%)	1.05
≥12 months	34 (61.8%)	2.91±2.349
D-Dimer (N=49)		
High	14 (28.5%)	3.93±2.710
Normal	35 (71.4%)	2.63±2.425
Fibrinogen (N=36)		
High	4 (11.1%)	1.95±1.743
Normal	32 (58.1%)	3.38±2.693
Platelet Aggregation Test		
Hypoaggregation	9 (16.3%)	1.13±0.708
Normoaggregation	21 (38.1%)	2.98±2.441
Hyperaggregation	25 (45.4%)	3.89±2.805
Thromboelastography (N=29)		
Normocoagulation	9 (31%)	2.85±2.501
Hypercoagulation	20 (68.9%)	3.50±2.719
Sinus Involvement		
Single	49 (89.1%)	2.72±2.358
Multiple	6 (10.9%)	6.08±2.681

Discussion

The median age of the study subjects was 48 years, with a range of 22–69 years, predominantly women. High estrogen levels in women of reproductive age who use hormonal contraceptives are known to induce a prothrombotic state through various mechanisms that affect the hemostatic pathway.¹⁵ The first mechanism, estrogen, modulates platelet activation

and aggregation and increases von Willebrand Factor (vWF) levels which have an important role in hemostasis.¹⁵ Venous thrombosis risk increases with oral contraceptives (OCs) use. Combined OCs containing estrogen and progesterone have higher risk. When the patient has a history of previous CVST, the recurrence risk is increased by OC use. In the subgroup analysis, a previous history of CVST was high in the “pregnancy group”. Very little is known about the relapse rate during pregnancy and puerperium in women with a history of CVST. The results of some study suggest that physicians must keep in mind the possible recurrence of CVST in pregnancy.¹⁶ In reproductive age, women, accompanied by other risk factors, especially genetic ones, will increase the likelihood of CVST.^{15,16}

The most frequent complaint was headache (92.7%). It is hypothesized that several mechanisms underlie the occurrence of headaches in CVST, namely intracranial hypertension, distention of veins and sinuses, and venous infarction.^{17–19} Clinical manifestations that appear in CVST patients can be according to the location of the affected sinus.²⁰ The most location of the affected sinuses in this study is in the transverse sinuses, as much as 70.9%, which will give symptoms of increased intracranial pressure, headache, hemianopia, contralateral hemiparesis, and aphasia if there is a cortical disturbance.²⁰

This study showed high P-selectin (>3.5 ng/mL) in 18 subjects. The majority of issues had normal P-selectin levels (0.95–3.5 ng/mL) in 25 subjects, and 12 subjects had low results (<0.95 ng/mL). In this study, 18 research subjects had increased P-selectin levels, seven subjects had unknown risk factors, five issues had risk factors for oral contraceptive use, and three had an infection and autoimmune risk factors. Of the 55 research subjects, there were 22 subjects with unknown risk factors due to the limitations of laboratory tests, such as examination of levels of Protein S, Protein C, and antiphospholipid, which are genetic risk factors related to hypercoagulation in CVST. The most risk factor was research subjects who consumed oral contraceptives, but the highest levels of P-selectin were found in the group with the infectious risk factor. The oral contraceptive user group has a 3 to 5 times higher risk of thrombotic events than the non-oral contraceptive group.^{21–23} The chance of thrombosis in oral contraceptive users depends on estrogen dose, progestin type, age, family history, and thrombophilic

conditions.²² The results of meta-analyses showed that gender-specific risk factors were only not effective in children and the elderly female groups and that the use of OCs increased venous thrombosis development in reproductive age females and thrombotic events most often occur in the first 12 months (especially the first three months) of oral contraceptive use, which is associated with exposure to new risk factors, especially the presence of other risk factors.^{16,21,22}

History of D-dimer examination showed 14 study subjects (28.5%) with high D-dimer levels and 35 study subjects (71.5%) with normal D-dimer levels. High P-selectin levels were also found in groups with high D-dimer values. An increase in D-dimer levels and P-selectin indicate activation of coagulation and fibrinolysis, which is also associated with increased thrombotic and recurrent thrombotic events.²⁴ D-dimer and P-selectin levels are grown in the acute phase and decreased significantly after one month of Deep Vein Thrombosis (DVT).²⁴ The combination of P-selectin, D-dimer, and total microparticle examinations had a sensitivity of 78% and a specificity of 81% for diagnosing DVT.²⁵ P-selectin has greater specificity compared to D-dimer and fibrinogen, namely 87% for predicting venous thrombosis events because it can specifically describe the hypercoagulable process that causes thrombus formation in veins.¹⁴ P-selectin levels can represent platelet activation in the early stages of thrombosis and as a direct inducer of procoagulant conditions associated with vascular and thrombotic events.^{7,10,11,13} No study has directly compared the D-dimer and P-selectin levels in CVST patients.

In this study, D-dimer and fibrinogen examinations were not carried out simultaneously with P-selectin tests but were carried out when the study subjects first came to the outpatient neurology polyclinic, when they were still in the acute and subacute phases. There were 14 study subjects with high D-dimer yields and four research subjects with high fibrinogen yields. The P-selectin examination was conducted when most study subjects (96.4%) were already in the chronic phase and had received anticoagulant or antiplatelet therapy. There were 18 study subjects with high P-selectin levels, with two issues in the subacute phase and 16 in the chronic phase. P-selectin levels can still increase in chronic phase CVST patients who have received anticoagulant or antiplatelet drugs. The P-selectin examination may help screen and diagnose more patients with hypercoagulable disorders or hyper platelet aggregation, in this case, CVST, when examined in the acute or subacute phase, so it can help patients to get appropriate and fast therapy. P-selectin may also be a tool for

evaluating therapy in patients currently receiving anticoagulant therapy and have reached the treatment target (INR 2–3) but have not experienced clinical improvement because P-selectin appears early in the thrombotic phase so that it will describe hypercoagulable conditions in CVST patients.

Limitations of Study

The following limitation should have been considered further in this study. First, the research subjects were patients diagnosed with CVST. They had received therapy, so this study did not show P-selectin levels in the acute phase, and the onset of P-selectin carried out was not homogenous. Second, many research subjects' risk factors still need to be discovered due to limited examinations. This study cannot describe all etiologies in CVST, especially genetic etiologies related to coagulation.

Conclusion

P-selectin levels were found to be increased in CVST patients with subacute and chronic onset, with the duration of treatment <3 Months, etiology of hormonal contraceptive use and multiple sinuses being affected. We need further study of P-selectin in acute onset CVST patients who have not received anticoagulant therapy and link it to genetic factors related to coagulation disorders. P-selectin could be a diagnostic marker for hyper aggregation and hypercoagulable state in patients with CVST, but it still needs further research to prove it.

Funding

No funding research.

Disclosure

There is no competing interest.

References

1. Stam J. Thrombosis of the cerebral veins and sinuses; 2005. Available from: www.nejm.org. Accessed May 29, 2023.
2. Ferro JM, Aguiar de Sousa D. Cerebral venous thrombosis: an update. *Curr Neurol Neurosci Rep*. 2019;19(10):1–9. doi:10.1007/s11910-019-0988-x
3. Boussier MG, Ferro JM. Cerebral venous thrombosis: an update. *Lancet Neurol*. 2007;6(2):162–170. doi:10.1016/S1474-4422(07)70029-7
4. Ferro JM, Canhão P, Aguiar de Sousa D. Cerebral venous thrombosis. *Press Med*. 2016;45(12):e429–e450. doi:10.1016/j.lpm.2016.10.007
5. Amalia L. The role of platelet-selectin as a marker of thrombocyte aggregation on cerebral sinus venous thrombosis. *J Blood Med*. 2022;13:267–274. doi:10.2147/JBM.S356028
6. Jellinger KA. Handbook of cerebral venous thrombosis. *Eur J Neurol*. 2009;16(2):e38–e38. doi:10.1111/j.1468-1331.2008.02469.x
7. Kozuka K, Kohriyama T, Ikeda J, Nakamura S, Nomura E, Kajikawa H. Endothelial markers and adhesion molecules in acute ischemic stroke-Sequential change and differences in stroke subtype. *Atherosclerosis*. 2002;161(1):161–168. doi:10.1016/S0021-9150(01)00635-9
8. Wagner DD. New links between inflammation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2005;25(7):1321–1324. doi:10.1161/01.ATV.0000166521.90532.44
9. Ramacciotti E, Blackburn S, Hawley AE, et al. Evaluation of soluble P-selectin as a marker for diagnosing deep venous thrombosis. *Clin Appl Thromb*. 2011;17(4):425–431. doi:10.1177/1076029611405032
10. André P. P-selectin in haemostasis. *Br J Haematol*. 2004;126(3):298–306. doi:10.1111/j.1365-2141.2004.05032.x
11. Antonopoulos CN, Sfyroeras GS, Kakisis JD, Moulakakis KG, Liapis CD. The role of soluble P selectin in the diagnosis of venous thromboembolism. *Thromb Res*. 2014;133(1):17–24. doi:10.1016/j.thromres.2013.08.014
12. Ferroni P, Martini F, Riondino S, et al. Soluble P-selectin as a marker of in vivo platelet activation. *Clin Chim Acta*. 2009;399(1–2):88–91. doi:10.1016/j.cca.2008.09.018
13. Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-I in coagulation and thrombosis. *Thromb Haemost*. 2004;92(3):459–466. doi:10.1160/TH04-05-0306
14. Fei Y, Zong GQ, Chen J, Liu RM. Evaluation of the value of d-dimer, P-selectin, and platelet count for prediction of portal vein thrombosis after devascularization. *Clin Appl Thromb*. 2016;22(5):471–475. doi:10.1177/1076029615569273
15. Abou-Ismaïl MY, Sridhar DC, Nayak L. Estrogen and thrombosis: A bench to bedside review. *Thromb Res*. 2020;192:40–51. doi:10.1016/j.thromres.2020.05.008.
16. Uluduz D, Sahin S, Duman T, et al. Cerebral venous sinus thrombosis in women: subgroup analysis of the VENOST study. *Stroke Res Treat*. 2020;2020. doi:10.1155/2020/8610903
17. Botta R, Donirpathi S, Yadav R, Kulkarni G, Kumar M, Nagaraja D. Headache patterns in cerebral venous sinus thrombosis. *J Neurosci Rural Pract*. 2017;8(5):72–77. doi:10.4103/jnpr.jnpr_339_16
18. Agostoni E. Headache in cerebral venous thrombosis. *Neurol Sci*. 2004;25(3):s206–s210. doi:10.1007/s10072-004-0287-3
19. Cumurciuc R, Crassard I, Sarov M, Valade D, Boussier MG. Headache as the only neurological sign of cerebral venous thrombosis: a series of 17 cases. *J Neurol Neurosurg Psychiatry*. 2005;76(8):1084–1087. doi:10.1136/jnnp.2004.056275

20. Saposnik G, Barinagarrementeria F, Brown RD, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42(4):1158–1192. doi:10.1161/STR.0b013e31820a8364
21. Saadatnia M, Tajmiriahi M. Hormonal contraceptives as a risk factor for cerebral venous and sinus thrombosis. *Acta Neurol Scand*. 2007;115(5):295–300. doi:10.1111/j.1600-0404.2007.00824.x
22. Trenor CC, Chung RJ, Michelson AD, et al. Hormonal contraception and thrombotic risk: a multidisciplinary approach. *Pediatrics*. 2011;127(2):347–357. doi:10.1542/peds.2010-2221
23. Sandset PM. Mechanisms of hormonal therapy related thrombosis. *Thromb Res*. 2013;131(1):S4–S7. doi:10.1016/S0049-3848(13)70009-4
24. Gremmel T, Ay C, Seidinger D, Pabinger I, Panzer S, Koppensteiner R. Soluble p-selectin, D-dimer, and high-sensitivity C-reactive Protein after acute deep vein thrombosis of the lower limb. *J Vasc Surg*. 2011;54(6):48S–55S. doi:10.1016/j.jvs.2011.05.097
25. Rectenwald JE, Myers DD, Hawley AE, et al. D-dimer, P-selectin, and microparticles: novel markers to predict deep venous thrombosis: a pilot study. *Thromb Haemost*. 2005;94(6):1312–1317. doi:10.1160/TH05-06-0426

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



A Critical Review of Sickle Cell Disease Burden and Challenges in Sub-Saharan Africa

Obi Peter Adigwe, Solomon Oloche Onoja & Godspower Onavbavba

To cite this article: Obi Peter Adigwe, Solomon Oloche Onoja & Godspower Onavbavba (2023) A Critical Review of Sickle Cell Disease Burden and Challenges in Sub-Saharan Africa, Journal of Blood Medicine, , 367-376, DOI: [10.2147/JBM.S406196](https://doi.org/10.2147/JBM.S406196)

To link to this article: <https://doi.org/10.2147/JBM.S406196>



© 2023 Adigwe et al.



Published online: 31 May 2023.



Submit your article to this journal



Article views: 2692



View related articles



View Crossmark data



Citing articles: 21 View citing articles

A Critical Review of Sickle Cell Disease Burden and Challenges in Sub-Saharan Africa

Obi Peter Adigwe¹, Solomon Oloche Onoja², Godspower Onavbavba¹

¹Office of the Director General, National Institute for Pharmaceutical Research and Development, Abuja, Federal Capital Territory, Nigeria;

²Department of Medical Laboratory Sciences, University of Nigeria, Enugu, Nigeria

Correspondence: Obi Peter Adigwe, Office of the Director General, National Institute for Pharmaceutical Research and Development, Abuja, Federal Capital Territory, Nigeria, Email o.p.adigwe@niprd.gov.ng

Abstract: Sickle cell disease is caused by an abnormality of the β -globin gene and is characterised by sickling of the red blood cells. Globally, sub-Saharan African countries share the highest burden of the disease. This study aimed at critically reviewing studies focusing on challenges of sickle cell anaemia in sub-Saharan Africa. A literature search was carried out in five major databases. Articles that met the inclusion criteria were included in the bibliometric review and critical analysis. A majority of the studies were undertaken in the West African region (85.5%), followed by Central Africa (9.1%). Very few studies had been undertaken in East Africa (3.6%), whilst the Southern African region had the fewest studies (1.8%). Distribution in relation to country revealed that three quarters of the studies were carried out in Nigeria (74.5%), followed by the Democratic Republic of the Congo (9.1%). According to healthcare settings, a strong majority of the studies were undertaken in tertiary health care facilities (92.7%). Major themes that emerged from the review include interventions, cost of treatment, and knowledge about sickle cell disease. Public health awareness and promotion as well as improving the quality of sickle cell centers for prompt management of patients with sickle cell disorder was identified as a critical strategy towards reducing the burden of the disease in sub-Saharan Africa. To achieve this, governments in countries located in this region need to adopt a proactive strategy in addressing gaps that have been identified in this study, as well as instituting other relevant measures, such as continuous media engagement and public health interventions relating to genetic counselling. Reforms in other areas that can help reduce the disease burden, include training of practitioners and equipping sickle cell disease treatment centers according to World Health Organization specifications.

Keywords: sickle cell anaemia, interventions, haemoglobin, genotype, genetics

Introduction

Sickle cell disease is an inherited autosomal recessive disorder of the β -globin gene characterised by clinical manifestations such as haemolytic anaemia and recurrent episodes of vascular occlusion.¹ Sickle cell disease refers to a group of inherited red blood cell disorders, with sickle cell anaemia indicated as one of the disorders. Disease presentation and progression in patients with sickle cell anaemia is highly variable. Individual differences in clinical presentations are also believed to be dependent on environment, the extent of sickling, vascular endothelium, platelets, leucocytes, and plasma proteins.²

Although sickle cell disease occurs worldwide, sub-Saharan Africa is the region with the highest prevalence.³ The disease is due to a mutation in the β -globin gene at chromosome 11 within the 17th nucleotide, where adenine replaces thymine. Consequently, glutamic acid is replaced by valine during translation, leading to the formation of abnormal haemoglobin. The abnormal haemoglobin formed is insoluble and polymerises with reduced oxygen tension, trauma, stress, dehydration, acidosis, or cold environments.⁴ Polymerised haemoglobin produces rigid, less flexible, and fragile erythrocytes with a reduced life span. These changes lead to various acute and chronic complications.⁵ Clinical presentations vary among sickle cell patients. Some include leg ulcers, priapism, fatigue, dizziness, osteonecrosis, bacteraemia, and dactylitis. Others are renal disease, pulmonary hypertension, acute chest syndrome, and end-organ damage.²

Sickle cell disorder has been acknowledged to have a global impact by the World Health Organization (WHO), with remarkable public health implications for Africa.⁶ Available evidence suggests 4.4 million people have sickle cell disease

worldwide, whilst about 43 million are living with sickle cell trait.⁷ About 80% of sickle cell disease cases occur in sub-Saharan Africa,⁸ and the mortality rate for children <5 years of age ranges from 50% to 80%.³ The high burden of the disease in this setting is further exacerbated due to lack of access to comprehensive healthcare in the region.⁹

Africa has been associated with the highest prevalence of the sickle cell trait, with figures suggesting that between 10% and 40% of the entire population may be affected.¹⁰ The incidence of sickle cell trait ranges from 20% to 30% in Cameroon, the Democratic Republic of the Congo, Gabon, Ghana, and Nigeria. A high prevalence of 45% has been reported in some parts of Uganda.¹¹ Available evidence suggests that about 90% of the world's sickle cell disease population lives in Nigeria, India, and the Democratic Republic of the Congo, where the disease affects up to 2% of the population.¹² Evidence suggests Nigeria has the largest population of persons affected with sickle cell disease globally.^{13,14}

Given the serious burden of sickle cell disease in sub-Saharan Africa, alongside inadequate measures to manage related crises arising from the condition, there is surprisingly little evidence in the extant literature that comprehensively critiques emergent issues associated with the condition. This study therefore aimed at critically reviewing studies that focused on challenges of sickle cell disease in sub-Saharan Africa.

Methods

We adopted a bibliometric review approach, with a critical analysis of the selected articles. This was done to enable a better understanding of the study area, which can then underpin further research, as well as guide policymakers in sub-Saharan Africa in developing new strategies to improve responses to sickle cell disease in the region. The review of the literature covered all full publications that appeared in English-language biomedical journals between 2003 and 2021. The search strategy included a combination of keywords: 'sickle cell disease', 'burden', 'interventions', 'challenges', and 'sub-Saharan Africa'. The literature search was carried out systematically using the PubMed, Scopus, Science Direct, and Google Scholar databases. Keywords and phrases were used to focus the search and to identify relevant articles. Following the search, titles and abstracts were reviewed and those articles that met the inclusion criteria were included in the review. The retrieved documents were examined to identify relevant articles. Articles considered were those that focused on sickle cell disease challenges, management, and interventions in sub-Saharan Africa. Only articles reporting primary and original research were included. Duplicates from various databases were eliminated. Data extracted from the selected articles were entered in Microsoft Excel 2016 for statistical analysis. The variables registered for each article were author, year of publication, journal title, design, and area of study. Others were healthcare setting, geographical region, sample and population of study, as well as outcomes. Descriptive statistical analysis was then undertaken, and results were summarised in figures.

Results

This bibliometric analysis and critical review of selected articles was targeted at better understanding relevant interventions, challenges of various intervention protocols, and identifying prospects for overcoming the burden of sickle cell disease in sub-Saharan Africa. The initial database search identified 1751 articles, published from 2003 to 2021. Following the elimination of duplicates as well as the application of the relevant screening and eligibility processes, a total of 55 articles were selected for critical review. Relevant details relating to the search, screening, and selection processes are presented in [Figure 1](#).

Bibliometry

Findings from this study revealed that investigations relating to sickle cell disease challenges and interventions had been undertaken in different healthcare settings. Various settings that emerged are presented in [Figure 2](#). A majority of the studies (92.6%) were undertaken in tertiary healthcare facilities. [Figure 2](#) reveals that there was a significant disparity between studies undertaken in tertiary facilities and other healthcare settings. [Figure 3](#) gives an overview of the geographical spread of studies on sickle cell disease. Findings showed that studies undertaken in the West African region (85.5%) dominated this category. This was followed by the Central African region (9.1%). It emerged that a majority of the studies were undertaken in West Africa, and few studies had been undertaken in other parts of sub-Saharan Africa.

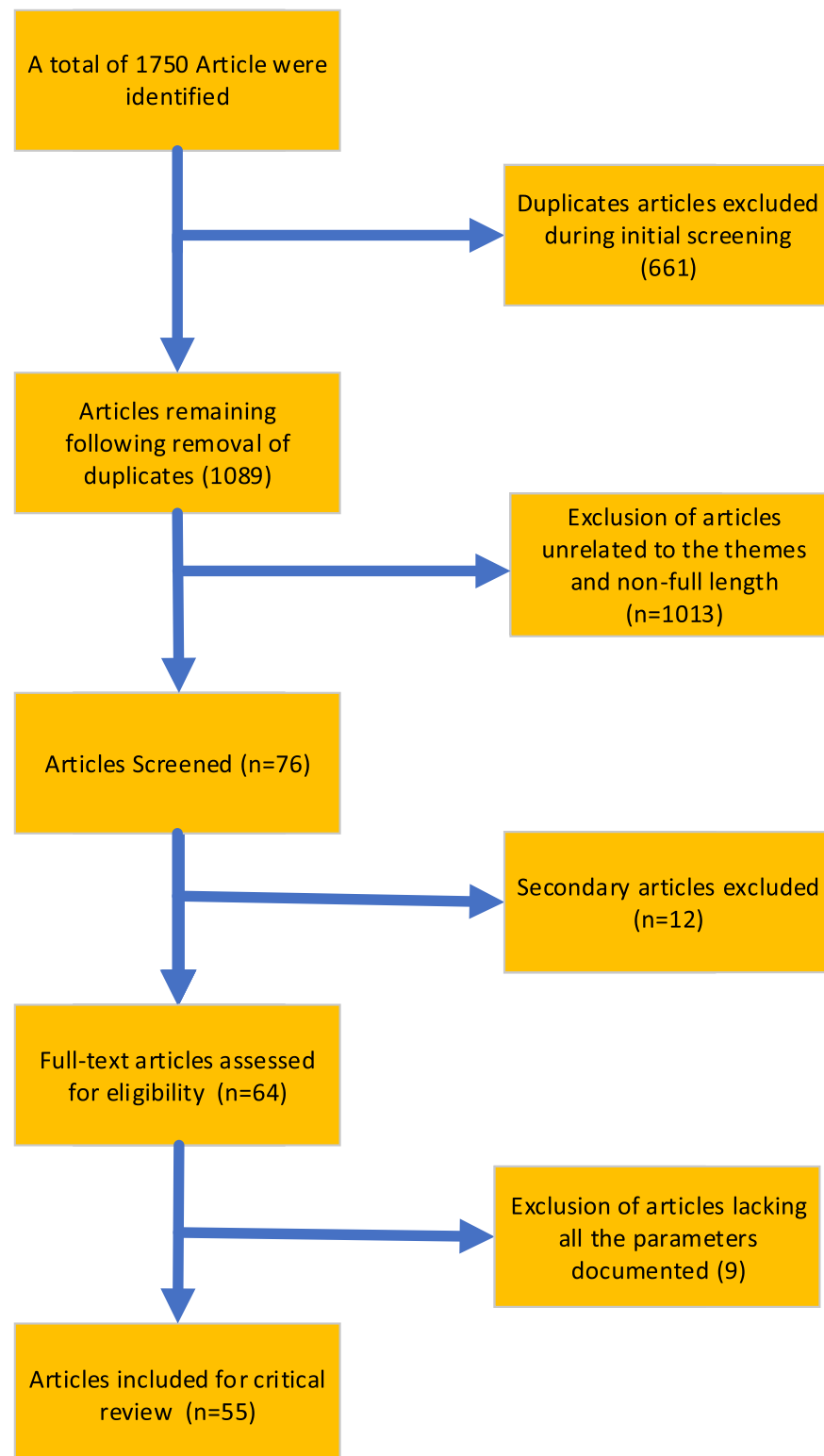


Figure 1 Review flow diagram.

Figure 4 provides details of the various articles included in the review according to country. It was observed that three quarters of the studies were undertaken in Nigeria (74.5%). The studies were further categorised according to year of publication. Those that were included in the review were published between 2003 and 2021. Findings in Figure 5 shows

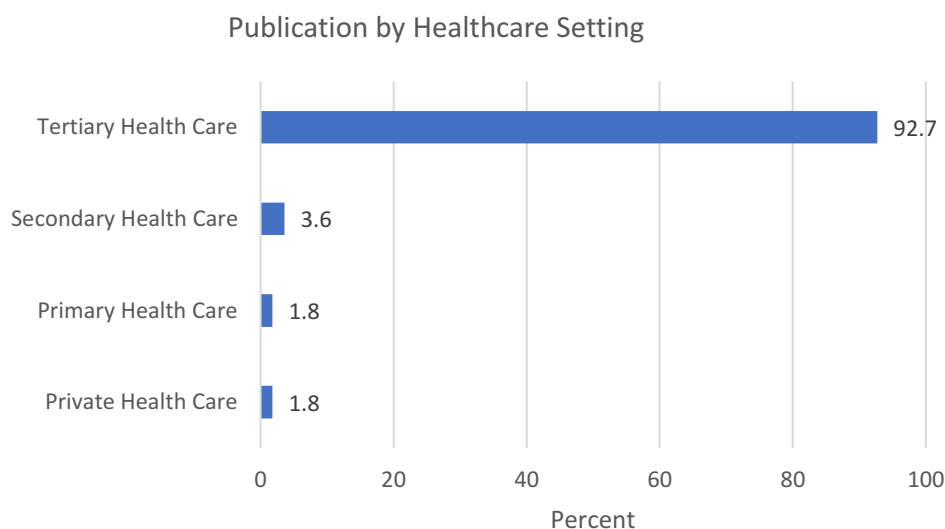


Figure 2 Distribution of studies based on healthcare settings.

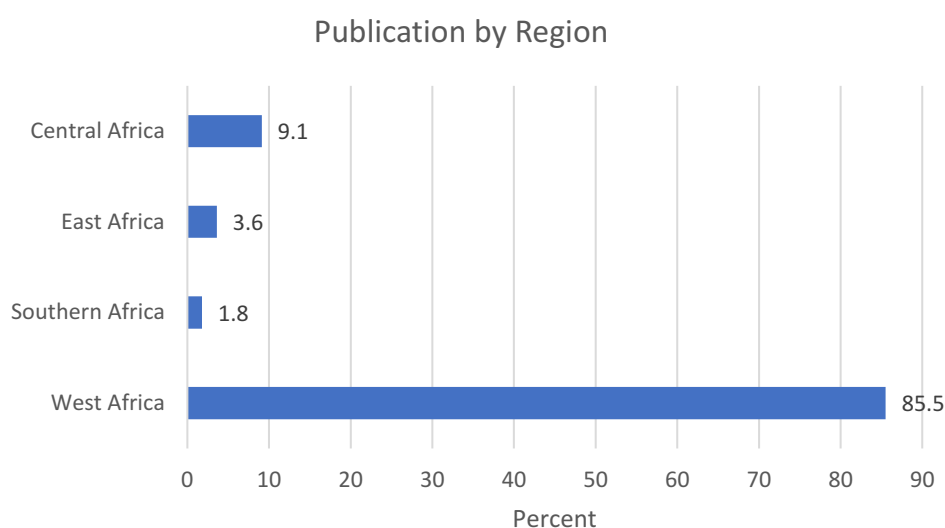


Figure 3 Geographic spread of studies.

that more studies were undertaken in 2020 (18.2%), and this was closely followed by 2019 (16.4%). Collectively, more than two thirds of the studies (69.1%) were carried out between 2017 and 2021, whilst less than a third were undertaken in 2003–2016. This shows an increase in research activity in this area in recent times.

Major Themes That Emerged

Therapeutic Interventions

Various articles explored therapeutic interventions for sickle cell disease in sub-Saharan Africa. A number of the articles centred on the potency and efficacy of hydroxyurea in the management of the condition in both children and adults. For instance, Ofakunrin et al¹⁵ studied the effectiveness and safety of hydroxyurea in the treatment of sickle cell anaemia among 54 children aged 4–17 years in Nigeria for 12 months, and they noted from their findings that the number of patients who had had more than two episodes of painful crises reduced drastically, whilst those with acute chest syndrome reduced to 0. Another study on hydroxyurea among 60 adults over 3 months also showed that the medicine has significant effect in ameliorating sickle cell disease.¹⁶ Hydroxyurea is currently approved for the management of

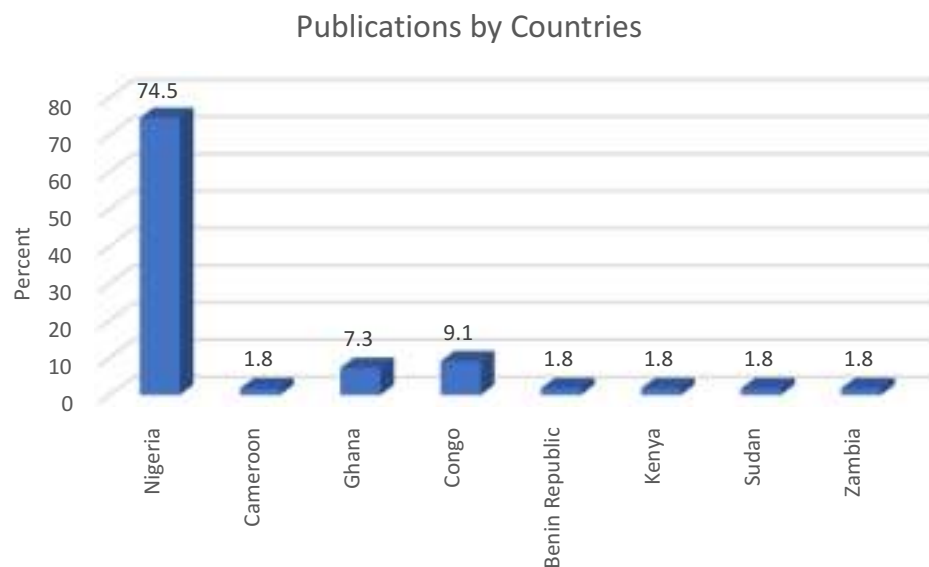


Figure 4 Publications by countries.

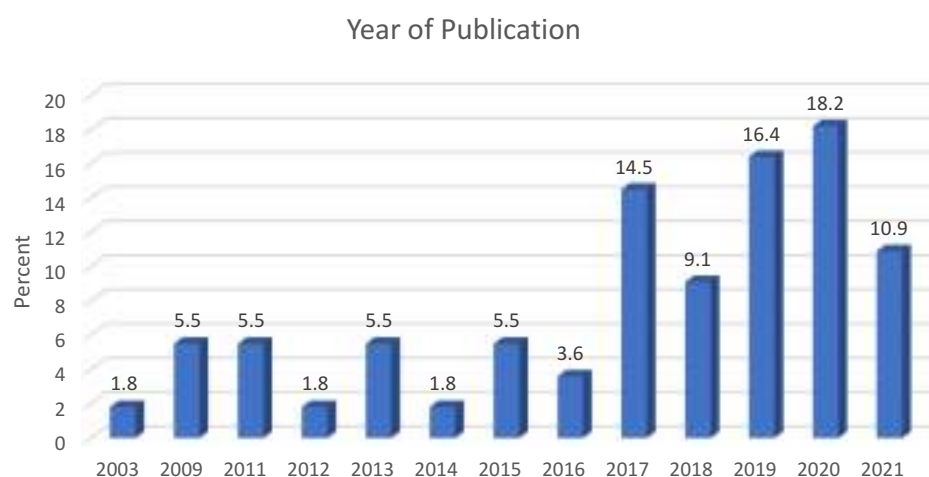


Figure 5 Distribution of studies according to year of publication.

sickle cell anaemia and it has contributed towards reducing the burden of the disease globally.¹⁷ A recent study demonstrated its safety and efficacy in children of 6–9 months of age.¹⁸ Another study also demonstrated the effectiveness of moderate fixed-dose hydroxyurea in primary stroke prevention, whilst another reported a decrease in the incidence of malaria episodes following hydroxyurea therapy.¹⁹ Furthermore, it was observed that the drug has robust efficacy and safety in the treatment of children with sickle cell anemia.²⁰ However, gaps identified in this area included short-term follow-up, inappropriate use of the intervention, and small samples.

Niprisan, which is now marketed as Niclovix, is a phytomedicine that has also been proven in recent times as a therapeutic intervention for sickle cell disease.^{21,22} It is an anti-sickling medicine extracted from local herbs in Nigeria. Niprisan was developed by the National Institute for Pharmaceutical Research and Development for the management of patients with sickle cell disease.²³ George et al evaluated the role of Niprisan among 115 children with sickle cell disease in Nigeria,²² and outcomes indicated a considerable reduction in hospital admissions from 47 (40.2%) to 13 (11.3%). It

was observed that Niprisan was effective in the management of sickle cell disease. Oniyangi and Cohall,²⁴ also reported safety and efficacy of the phytomedicinal intervention in their study.

A survey on the effect of a comprehensive clinical care programme on severely ill patients suggested marked reduction in the frequency and severity of sickle cell-related acute events, with improvement in general status and physical growth among 184 patients (78%).²⁵ Taking into account the social, cultural, and economic backgrounds of sub-Saharan Africa, provision of information and education about the disease to affected persons has proven to be effective in reducing challenges associated with the condition.²⁶

Challenges associated with suboptimal management had been reported. In their study on acute pain management in children with sickle cell anaemia during emergency admission, Oshikoya et al²⁷ highlighted inadequate management of pain with analgesics. The study also identified that pain management among children was not in conformity with the guidelines of the World Health Organization.

A study undertaken in the Democratic Republic of the Congo on the management of sickle cell disease also highlighted insufficient standard-care practices for patients in Kisangani.²⁸ Findings from this study indicated that comprehensive management of sickle cell disease, including specific interventions, such as neonatal screening, early diagnosis, preventive penicillin therapy, pneumococcal and haemophilic influenza vaccination, and malaria prophylaxis, were all lacking. Mukinayi et al²⁹ highlighted poor diagnostic and treatment options as factors contributing to increased morbidity and mortality for sickle cell anaemia patients in the Democratic Republic of the Congo. Galadanci et al¹⁴ reported similar findings in their study on current sickle cell disease management practices in Nigeria.

Knowledge About Sickle Cell Disease

Various studies in this review identified knowledge deficits about the haemoglobin genotype, premarital genetic counseling, and screening in sub-Saharan Africa. These gaps seemed to have contributed to the prevalence of the condition. A cross-sectional study by Obed et al³⁰ revealed knowledge deficits about sickle cell disease among Ghanaian pregnant women, and a limited understanding of sickle cell disease among university students, particularly on the pattern of inheritance, was reported by Boadu and Addoah.³¹ Similar findings were reported from studies among Kisangani University students,³² families directly affected by sickle cell disease in Mbuji-Mayi,³³ and among healthcare providers in the city of Kindu, all in the Democratic Republic of the Congo.^{28,34} Poor knowledge about sickle cell disease was identified amongst unmarried youths in Cameroon.³⁵ In Nigeria, poor knowledge about sickle cell disease has also been reported among secondary school students,^{36,37} undergraduates,^{1,38,39} local government workers,⁴⁰ and childbearing-age adult women.^{41–43} Level of education has been reported as a determinant of good knowledge about sickle cell disease.^{44,45}

A cross-sectional survey by Rasheed et al⁴⁶ on married and unmarried youths in Nigeria revealed that the level of knowledge of participants was good (70.06%); however, participants had negative attitudes towards the practice of premarital sickle cell genetic screening. The findings from that study were attributed to inadequate public health awareness and lack of basic knowledge on the haemoglobin genotype and sickle cell disease. Similar findings were reported among some secondary school students in Nigeria,^{47–49} as well as tertiary institutions.^{50–52}

On the contrary, several studies revealed adequate knowledge of sickle cell disease, genetic counselling, and haemoglobin genotyping with positive preventive practices and attitudes towards the condition. Significant knowledge of the causes, signs, and symptoms of sickle cell disease with positive preventive practices towards the condition was reported among students of tertiary institutions^{53–55} and youths in Yaba, Nigeria.^{56,57} Oluwole and Adeyemo⁵⁸ reported high levels of awareness, good knowledge, and positive attitudes towards sickle cell disease. A majority of respondents in that study (84.9%) were also willing to screen their newborns for sickle cell disease. Good knowledge and positive attitudes were observed among religious and traditional leaders in Nigeria.⁵⁹ In Ghana, studies among women⁶⁰ and young unmarried adults revealed positive attitudes towards genetic testing and counselling and practices towards sickle cell disease.⁶¹ Similar findings were reported in Zambia.⁶²

Cost of Intervention

Public perceptions relating to the cost of management of sickle cell disease have also been explored in the extant literature. In a study that explored factors relating to the financial burden of sickle cell disease in Nigeria, the findings

showed that few participants (7.2%) were enrolled in the National Health Insurance Scheme, with out-of-pocket payments on the increase.⁶³ Monthly household income ranged from ₦12,500 (US\$27.15) to ₦330,000 (\$716.64), whilst health expenditure was between ₦2500 (\$5.43) and ₦215,000 (\$466.90). The high financial burden exposed participants to financial hardship due to expenses and loans incurred in order to offset hospital bills and other relevant healthcare costs. Health insurance was however reported as an invaluable tool that can help ameliorate financial burden associated with the management of sickle cell anaemia.⁶³ Another study explored the cost of treatment in Kenya, and the high cost of treatment was also reported.⁶⁴ High cost of diagnosis was similarly identified as a factor limiting prenatal screening for sickle cell disease.^{65,66}

Discussion

Various studies relating to the challenges and burden of sickle cell disease have been undertaken in sub-Saharan Africa. A majority of the studies carried out were in tertiary health care centres. Whilst it is understandable that tertiary health care facilities seem to be a familiar setting for such studies, this finding reveals a paucity of information from other settings. Ordinarily, primary healthcare centres should serve as the first point of contact when seeking medical intervention.⁶⁶ It appears, however, that primary and secondary health facilities have not received the needed attention as it relates to challenges of sickle cell disease. One reason for this disparity could be attributed to the nature of tertiary health facilities, which combine both healthcare delivery and academic activities.

Findings from this study also revealed disparity between the West African region, where a significant proportion of the studies were carried out, compared to the much smaller proportions from other regions in sub-Saharan Africa. The high proportion of studies from the West African region could be associated with the high burden of sickle cell disease in the region.³ In the Central African region, only the Democratic Republic of the Congo had considerable research activity relating to this subject area, whilst only Kenya and Sudan had undertaken such studies in the East African region. Furthermore, the classification of the articles according to country revealed that three-quarters of the studies were undertaken in Nigeria, and this may be perhaps due to the high prevalence of sickle cell disease in the country compared to other nations.^{13,14} The findings of this study not only provide a basis for further studies that can address the knowledge gaps but they also clearly reveal the urgent need for a review of research and development strategies that can better contribute to the control and eradication of the disease.

Sickle cell disease remains a public health challenge in sub-Saharan Africa.⁶⁷ Findings from this study suggest that various intervention efforts such as the use of hydroxyurea in managing the condition as well as the development of Niprisan have yielded significant result in reducing the burden of the disease. Hydroxyurea changes the complex pathophysiology of sickle cell anaemia through such mechanisms as improvement of cellular hydration, induction of fetal haemoglobin production, reduction of leucocyte and platelet counts, and production of nitric oxide. Clinically, this translates to reduced frequency and intensity of sickle cell crisis, hospitalisations, and blood transfusions, with holistic improvement in the quality of life of sickle cell patients.^{15,16,68} However, inappropriate use of hydroxyurea was observed in this review. This can consequently affect clinical outcomes despite its robust benefits and proven efficacy in the management of sickle cell disease. There is a need for training and retraining of healthcare professionals on the use and safety of hydroxyurea, Niprisan and other relevant products for the management of sickle cell disease so as to improve clinical outcomes. Findings from this study also highlight the need for the prioritisation of phytomedicine development in sub-Saharan Africa, as Niprisan, which was developed from four plants, was found to be efficacious in the management of the disease.^{21–24,69}

This study further revealed that interventions with respect to timely diagnosis were inadequate. This suggests the need to scale up point-of-care testing as well as infant screening for early identification and management of individuals affected with the disorder so as to prevent unnecessary loss of lives.⁷⁰ Other factors that contribute to the high prevalence of sickle cell anaemia in sub-Saharan Africa include poor knowledge about the condition. Therefore, developing capacity in public health awareness, genetic counselling, and proper premarital education can serve as vital means of reducing the prevalence of the disease.⁷¹ Keeping citizens adequately informed about the condition is key to reducing the burden of the disease in sub-Saharan Africa. In addition, the inclusion of topics relating to sickle cell disease in educational curricula, especially at an early stage, can help improve awareness about the condition at a young age. Improved strategic

collaboration between the ministries of health and education can play a critical role in addressing some of these concerns. Improving awareness at the grassroots level can be amplified and expedited through community-based structures such as cultural and religious groups.

Findings from this study suggest the management of sickle cell disease is associated with high financial burden and the payment for healthcare services were mainly out-of-pocket spending. Based on these findings, there is a need for governments in countries with high prevalence of the disease to come up with a robust and comprehensive policy that can alleviate the high cost of care associated with managing the condition. Such an approach can help improve the quality of health for patients with sickle cell disorder.^{64,65}

Conclusion

This study adopted a novel critical review strategy to appraise the body of work undertaken in sickle cell disease over approximately two decades. Findings highlighted various challenges in the current management of the disease, as well as the importance of increasing efforts towards public enlightenment about the disease so as to increase awareness.

The gaps from the holistic review also underscore the need for countries across sub-Saharan Africa to equip treatment centers with adequate facilities as well as to regularly train healthcare personnel with respect to the latest techniques for prevention, management and treatment of the disease. The need for adherence to standard sickle cell disease management protocol at all levels also emerged as an important strategy to reduce the burden associated with the disease. Despite the criticality of primary health centres in healthcare provision, several of these facilities across sub-Saharan African countries seemed underutilised, suggesting necessary reforms, revitalisation, and adequate equipping to enhance their utility in sickle cell prevention and care.

Given the high prevalence of sickle cell disease in sub-Saharan Africa, there is an urgent need for governments and relevant stakeholders in this region to prioritise strategies that can underpin effective management of the disease. In addition to managing the disease, novel findings that emerged due to the unique methodology adopted can enable public health synergies that will reduce the burden whilst targeting total elimination. Genetic counselling, premarital genotype screening, and comprehensive health insurance coverage for all sickle cell disease patients are some key elements in strategic reforms that can improve access to care whilst also contributing to an overarching reduction of the disease burden.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ugwu NI. Sickle cell disease: awareness, knowledge and attitude among undergraduate students of a Nigerian tertiary educational institution. *Asian J Med Sci.* 2016;7(5):87–92. doi:10.3126/ajms.v7i5.15044
2. Onoja SO, Eluke BC, Dangana A, Musa S, Abdullah IN. Evaluation of von Willebrand factor and other coagulation homeostasis profile of patients with sickle cell anemia attending a tertiary hospital at Enugu, Nigeria. *Med J Zambia.* 2020;47(4):269–275. doi:10.55320/mjz.47.4.715
3. Aliyu ZY, Kato GJ, Taylor J, et al. Sickle cell disease and pulmonary hypertension in Africa: a global perspective and review of epidemiology, pathophysiology, and management. *Am J Hematol.* 2008;83(1):63–70. doi:10.1002/ajh.21057
4. De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. In: *Seminars in thrombosis and hemostasis.* Thieme Med Pub. 2011;37(3):226–236. doi:10.1055/s-0031-1273087
5. Malowany JI, Butany J. Pathology of sickle cell disease. *Semin Diagn Pathol.* 2012;29(1):49–55. doi:10.1053/j.semmp.2011.07.005
6. Hsu L, Nnodu OE, Brown BJ, et al. White paper: pathways to progress in newborn screening for sickle cell disease in Sub-Saharan Africa. *J Trop Dis Public Health.* 2018;6(2):260. doi:10.4172/2329-891X.1000260
7. Global Burden of Disease Study. Collaborators (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2013;386(9995):743–800. doi:10.1016/s0140-6736(15)60692-4
8. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet.* 2010;376(9757):2018–2031. doi:10.1016/s0140-6736(10)61029-x
9. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet.* 2013;381(9861):142–151. doi:10.1016/S0140-6736(12)61229-X
10. Agasa B, Bosunga K, Opara A, et al. Prevalence of sickle cell disease in a northeastern region of the Democratic Republic of Congo: what impact on transfusion policy? *Transfus Med.* 2010;20(1):62–65. doi:10.1111/j.1365-3148.2009.00943.x
11. Afolayan JA, Jolayemi FT. Parental attitude to children with sickle cell disease in selected health facilities in Irepodun Local Government, Kwara State, Nigeria. *Stud Ethno Med.* 2011;5(1):33–40. doi:10.1080/09735070.2011.11886389
12. Kadima BT, Gini Ehungu JL, Ngiyulu RM, Ekulu PM, Aloni MN. High rate of sickle cell anaemia in S ub-S aharan A frica underlines the need to screen all children with severe anaemia for the disease. *Acta Paediatr.* 2015;104(12):1269–1273. doi:10.1111/apa.13040

13. Fleming AF, Storey J, Molineaux L, Iroko EA, Attai ED. Abnormal haemoglobins in the Sudan savanna of Nigeria: i. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. *Ann Trop Med Parasitol.* 1979;73(2):161–172. doi:10.1080/00034983.1979.11687243
14. Galadanci N, Wudil BJ, Balogun TM, et al. Current sickle cell disease management practices in Nigeria. *Int Health.* 2014;6(1):23–28. doi:10.1093/inthealth/ih022
15. Ofakunrin AO, Oguche S, Adekola K, et al. Effectiveness and safety of hydroxyurea in the treatment of sickle cell anaemia children in Jos, North Central Nigeria. *J Trop Pediatr.* 2020;66(3):290–298. doi:10.1093/tropej/fmz070
16. Adewoyin AS, Oghuvwu OS, Awodu OA. Hydroxyurea therapy in adult Nigerian sickle cell disease: a monocentric survey on pattern of use, clinical effects and patient's compliance. *Afr Health Sci.* 2017;17(1):255–261. doi:10.4314/ahs.v17i1.31
17. Halsey C, Roberts IA. The role of hydroxyurea in sickle cell disease. *Br J Haematol.* 2003;120(2):177–186. doi:10.1046/j.1365-2141.2003.03849.x
18. Thornburg CD, Files BA, Luo Z, et al. Impact of hydroxyurea on clinical events in the BABY HUG trial. *J Am Soc Hematol.* 2012;120(22):4304–4310. doi:10.1182/blood-2012-03-419879
19. Galadanci NA, Umar Abdullahi S, Vance LD, et al. Feasibility trial for primary stroke prevention in children with sickle cell anemia in Nigeria (SPIN trial). *Am J Hematol.* 2017;92(8):780–788. doi:10.1002/ajh.24770
20. Tshilolo L, Tomlinson G, Williams TN, et al. Hydroxyurea for children with sickle cell anemia in sub-Saharan Africa. *N Engl J Med.* 2019;380(2):121–131. doi:10.1056/NEJMoa1813598
21. Wambebe CO, Bamgboye EA, Badru BO, et al. Efficacy of nuprisan in the prophylactic management of patients with sickle cell disease. *Curr Ther Res.* 2001;62(1):26–34. doi:10.1016/S0011-393X(01)80039-4
22. George IO, Frank-Briggs A, Odigie JO. Nicosan therapy: any role in children with sickle cell anemia in Nigeria. *Int J Trop Med.* 2011;6(6):121–123.
23. Iyamu EW, Turner EA, Asakura T. In vitro effects of NIPRISAN (Nix-0699): a naturally occurring, potent antisickling agent. *Br J Haematol.* 2002;118(1):337–343. doi:10.1046/j.1365-2141.2002.03593.x
24. Oniyangi O, Cohall DH. Phytomedicines (medicines derived from plants) for sickle cell disease. *Cochrane Database Syst Rev.* 2020;9. doi:10.1002/14651858.CD004448.pub7
25. Rahimy MC, Gangbo A, Ahouignan G, Adjou R, Deguenon C, Goussanou S. Effect of a comprehensive clinical care program on disease course in severely ill children with sickle cell anemia in a sub-Saharan African setting. *Blood.* 2003;102:834–838. doi:10.1182/blood-2002-05-1453
26. Ohaeri JU, Shokunbi WA. Psychosocial burden of sickle cell disease on caregivers in a Nigerian setting. *J Natl Med Assoc.* 2002;94(12):1058–1070.
27. Oshikoya KA, Edun B, Oreagba IA. Acute pain management in children with sickle cell anaemia during emergency admission to a teaching hospital in Lagos, Nigeria. *S Afr J Child Health.* 2015;9(4):119–123. doi:10.7196/SAJCH.2015.v9i4.968
28. Kambale-Kombi P, Marini Djang'eing'a R, Alworong'a Opara JP, Tonen-Wolyec S, Kayembe Tshilumba C, Batina-Agasa S. Students' knowledge on sickle cell disease in Kisangani, Democratic Republic of the Congo. *Hematology.* 2020;25(1):91–94. doi:10.1080/16078454.2020.1727174
29. Mukinayi MB, Tumba DG, Gulbis B. Sickle cell disease in the democratic republic of Congo: assessing physicians' knowledge and practices. *Trop Med Infect Dis.* 2020;5(3):127. doi:10.3390/tropicalmed5030127
30. Obed SA, Asah-Opoku K, Aboagye S, Torto M, Oppong SA, Nuamah MA. Awareness of sickle cell trait status: a cross-sectional survey of antenatal women in Ghana. *Am J Trop Med Hyg.* 2017;96(3):735–740. doi:10.4269/ajtmh.16-0396
31. Boadu I, Addoah T. Knowledge, beliefs and attitude towards sickle cell disease among university students. *J Community Med Health Educ.* 2018;8(1):1000593. doi:10.4172/2161-0711.1000593
32. Kambale-Kombi P, Marini Djang'eing'a R, Alworong'a Opara JP, et al. Management of sickle cell disease: current practices and challenges in a northeastern region of the Democratic Republic of the Congo. *Hematology.* 2021;26(1):199–205. doi:10.1080/16078454.2021.1880752
33. Mukinayi BM, Kalenda DK, Mbelu S, Gulbis B. Awareness and attitudes of 50 Congolese families affected by sickle cell disease: a local survey. *Pan Afr Med J.* 2018;29:24. doi:10.11604/pamj.2018.29.24.12276
34. Katawandja AL. Knowledge and practices of health providers on the diagnosis and biological monitoring of sickle cell disease in the City of Kindu, in the East of the Democratic Republic of Congo. *Open Access Libr J.* 2020;7:e6757. doi:10.4236/oalib.1106757
35. Ngwengi NY, Fon PN, Mbanya D. Distribution of haemoglobin genotypes, knowledge, attitude and practices towards sickle cell disease among unmarried youths in the Buea Health District, Cameroon. *Pan Afr Med J.* 2020;37:109. doi:10.11604/pamj.2020.37.109.17864
36. Bazuaye GN, Olayemi EE. Knowledge and attitude of senior secondary school students in Benin City Nigeria to sickle cell disease. *World J Medical Sci.* 2009;4(1):46–49.
37. Olakunle OS, Kenneth E, Olakekan AW, Adenike OB. Knowledge and attitude of secondary school students in Jos, Nigeria on sickle cell disease. *Pan Afr Med J.* 2013;15(1). doi:10.11604/pamj.2013.15.127.2712
38. Hussaini MA, Durbunde AA, Jobbi YD, et al. Assessment of experience, perception and attitude towards premarital sickle cell disease screening among students attending federal college of education, Kano, Nigeria. *Int J Res Rep Hematol.* 2019;4:1–2. doi:10.11604/pamj.2021.38.350.20894
39. Alao OO, Araoye M, Ojabo C. Knowledge of sickle cell disease and haemoglobin electrophoresis: a survey of students of a tertiary institution. *Niger J Med.* 2009;18(3):326–329. doi:10.4314/njm.v18i3.51208
40. Abioye-Kuteyi EA, Osakwe C, Oyegbade O, Bello I. Sickle cell knowledge, premarital screening and marital decisions among local government workers in Ile-Ife, Nigeria. *Afr J Prim Health Care Fam Med.* 2009;1(1):1–5. doi:10.4102/phcfm.v1i1.22
41. Otovwe A, Sunday UI, Oghenenioborue Rume OB, Awulo DM. Knowledge and attitude of premarital genotype screening among women of child-bearing age in kumo-akko local government area of Gombe State Nigeria. *Open J Public Health.* 2019;1(2):1006.
42. Babalola OA, Chen CS, Brown BJ, Cursio JF, Falusi AG, Olopade OI. Knowledge and health beliefs assessment of sickle cell disease as a prelude to neonatal screening in Ibadan, Nigeria. *J Glob Health Rep.* 2019;3:e2019062. doi:10.29392/joghr.3.e2019062
43. Agofure O, Danzaria MA. Knowledge and attitude towards premarital genotype screening among women of child-bearing age in kumo akko local government area of Gombe State. *Open J Med Res.* 2020;1(1):10–19. doi:10.52417/ojmr.v1i1.73
44. Owolabi RS, Alabi P, Olusoji D, Ajayi S, Otu T, Ogundiran A. Knowledge and attitudes of secondary school students in Federal Capital Territory (FCT), Abuja, Nigeria towards sickle cell disease. *Niger J Med.* 2011;20(4):479–485.
45. Daak AA, Elsamani E, Ali EH, et al. Sickle cell disease in western Sudan: genetic epidemiology and predictors of knowledge attitude and practices. *Trop Med Int Health.* 2016;21(5):642–653. doi:10.1111/tmi.12689
46. Rasheed TO, Afolabi WA, Abdul Rasheed RO, Ajala RA. Premarital sickle cell genetic screening knowledge, attitude and practice compared among married and unmarried youths in Nigeria. *World J Public Health.* 2018;3(3):76–82. doi:10.11648/j.wjph.20180303.12

47. Oluwadamilola AD, Akinreni TI, Adefisan MA, Olayiwola SD. Knowledge, attitude and control practices of sickle cell diseases among senior secondary students in Osun State, Nigeria. *Pan Afr Med J.* 2021;38:350. doi:10.11604/pamj.2021.38.350.20894
48. Abdulhameed A, Yalma RM. Knowledge, attitude, and practice of genotype screening among junior and senior secondary school students in gwagwalada area council, FCT Abuja. *Int J Prog Sci Technol.* 2021;26(2):272–280.
49. Ezenwosu OU, Chukwu BF, Ezenwosu IL, Ikefuna AN, Emodi IJ, Ezeanolue EE. Knowledge and awareness of individual sickle cell genotype among adolescents in a unity school in Southeast, Nigeria: a cross-sectional study. *Int J Adolesc Med Health.* 2020;20190149. doi:10.1515/ijamh-2019-0149
50. Faremi AF, Olatubi IM, Lawal YR. Knowledge of sickle cell disease and pre marital genotype screening among students of a tertiary educational institution in south western Nigeria. *Int J Caring Sci.* 2018;11(1):285–295.
51. Uche E, Olowoselu O, Augustine B, et al. An assessment of knowledge, awareness, and attitude of undergraduates toward sickle cell disease in Lagos, Nigeria. *Niger J Med.* 2017;58(6):167. doi:10.4103/nmj.NMJ_111_18
52. Bosah CN, Alagbu CE. Knowledge and attitude of Nnamdi Azikiwe University undergraduate students towards pre-marital sickle cell screening. *J Early Child Prim Educ.* 2020;2(1):87–99.
53. Chukwurah EF, Oduma FC, Madubuattah CG, Chukwurah FC. Assessment of knowledge and attitude of sickle cell genetic screening among fresh undergraduate students of Ebonyi State University, NigeriaAbakaliki. *J Med Lab Sci.* 2019;29(3):8–20.
54. Kehinde ME, Sowunmi C. Sickle cell disease knowledge, premarital genotype screening and marital decision among unmarried students of Lagos state Polytechnic Ikorodu, Lagos, Nigeria. *EASIJ.* 2021;3(5):53–65. doi:10.5281/zenodo.4768648
55. Yalma RM, Awodiji MM. Knowledge, attitude and practice of genotype screening among undergraduate students of the university of Abuja, Nigeria. *Eur J Prev Med.* 2021;9(2):63–70. doi:10.11648/j.ejpm.20210902.16
56. Oludare GO, Ogili MC. Knowledge, attitude and practice of premarital counseling for sickle cell disease among youth in Yaba, Nigeria. *Afr J Reprod Health.* 2013;17(4):175–182.
57. Adegbite OA. Young people's knowledge of sickle cell disease and willingness for genotype screening in Ibadan, Nigeria. *Afr J Biomed Res.* 2021;24(2):211–217.
58. Oluwale EO, Adeyemo TA. Knowledge, attitude and willingness to screen younger infants for sickle cell disease among mothers attending immunization clinic in an urban community in Lagos, Nigeria. *J Comm Med Prim Health Care.* 2021;33(2):52–67. doi:10.4314/jcmphc.v33i2.4
59. Abubakar SB, Abdulqadir I, Magaji BA, Sanusi N, Ibrahim F, Maiturare M. Knowledge, attitude and perception of traditional and religious leaders on pre-marital screening for sickle cell disease in Sokoto. *Int J Med Public Health.* 2019;9(2):36–41. doi:10.5530/ijmedph.2019.2.10
60. Ross PT, Lypson ML, Ursu DC, Everett LA, Rodrigues O, Campbell AD. Attitudes of Ghanaian women toward genetic testing for sickle cell trait. *Int J Gynaecol Obstet.* 2011;115(3):264–268. doi:10.1016/j.ijgo.2011.08.004
61. Appiah S, Korsah KA, AmpongAdjei C, Appiah OE. Genetic counselling in sickle cell disease: views of single young adults in Ghana. *J Community Genet.* 2020;11(4):485–493. doi:10.1007/s12687-020-00474-4
62. Nakazwe E, Mwanakasale V, Siziya S. Knowledge attitude and practices of parents with children suffering from sickle cell disease towards factors that precipitate sickle cell crises, at arthur davidson children's hospital in Ndola Zambia. *Asian Pac J Health Sci.* 2017;18:18–27. doi:10.21276/apjhs.2017.4.3.26
63. Olatunya OS, Ogundare EO, Fadare JO, et al. The financial burden of sickle cell disease on households in Ekiti, Southwest Nigeria. *CEOR.* 2015;7:545. doi:10.2147/CEOR.S86599
64. Amendah DD, Mukamah G, Komba A, Ndila C, Williams TN. Routine paediatric sickle cell disease (SCD) outpatient care in a rural Kenyan hospital: utilization and costs. *PLoS One.* 2013;8(4):e61130. doi:10.1371/journal.pone.0061130
65. Olatunya OS, Babatola AO, Ogundare EO, et al. Perceptions and practice of early diagnosis of sickle cell disease by parents and physicians in a southwestern state of Nigeria. *Sci World J.* 2020;2020:4801087. doi:10.1155/2020/4801087
66. Nnodu OE, Adegoke SA, Ezenwosu OU, et al. A multi-centre survey of acceptability of newborn screening for sickle cell disease in Nigeria. *Cureus.* 2018;10(3). doi:10.7759/cureus.2354
67. Akinbami A, Dosunmu A, Adediran A, Oshinaike O, Adebola P, Arogundade O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Res Notes.* 2012;5(1):1–6. doi:10.1186/1756-0500-5-396
68. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. *J Am Soc Hematol.* 2010;115(26):5300–5311. doi:10.1182/blood-2009-04-146852
69. Adzu B, Masimirembwa C, Mustapha KB, Thelingwani R, Kirim RA, Gamaniel KS. Effect of NIPRISAN® on CYP3A4 activity in vitro. *Eur J Drug Metab Pharmacokinet.* 2015;40(1):115–118. doi:10.1007/s13318-014-0173-1
70. Nnodu O, Isa H, Nwegbu M, et al. HemoType SC, a low-cost point-of-care testing device for sickle cell disease: promises and challenges. *Blood Cells Mol Dis.* 2019;78:22–28. doi:10.1016/j.bcmd.2019.01.007
71. Olatona FA, Odeyemi KA, Onajole AT, Asuzu MC. Effects of health education on knowledge and attitude of youth corps members to sickle cell disease and its screening in Lagos State. *J Community Med Health Educ.* 2012;2:163. doi:10.4172/2161-0711.1000163

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines [Letter]

Anumta Ali, Syeda Adeena Zafar & Syeda Sakina Zehra

To cite this article: Anumta Ali, Syeda Adeena Zafar & Syeda Sakina Zehra (2023) Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines [Letter], Journal of Blood Medicine, , 377-378, DOI: [10.2147/JBM.S419849](https://doi.org/10.2147/JBM.S419849)

To link to this article: <https://doi.org/10.2147/JBM.S419849>



© 2023 Ali et al.



Published online: 02 Jun 2023.



Submit your article to this journal



Article views: 208



View related articles



View Crossmark data

Immune Thrombocytopenia Relapse in Patients Who Received mRNA COVID-19 Vaccines [Letter]

Anumta Ali, Syeda Adeena Zafar, Syeda Sakina Zehra 

Karachi Medical and Dental College, Karachi, Pakistan

Correspondence: Anumta Ali, Tel +92 3128831575, Email alianumta1@gmail.com

Dear editor

We have read the research conducted by Hana Qasim et al¹ with great interest. We appreciate the author for sharing her experience and would like to share our outlook which has raised a few questions in our mind.

The authors selected 67 patients for this study; however, it is not mentioned whether any of them had gone through the previous surgery of splenectomy. As splenectomy is the treatment of choice for ITP if the patient becomes refractory to the conventional treatment, so post-vaccination relapse of ITP symptoms even after splenectomy is a big question mark for our diagnosis. Further age-related comorbidities should be discussed such as diabetes mellitus, cataract, GI disease, and some dermatological conditions which have direct association with ITP.²

The effect of the vaccine on the level of thrombopoietin should have been highlighted. Previous studies have shown that antibodies that can cross-react with thrombopoietin can induce thrombocytopenia. This is due to molecular mimicry between the human thrombopoietin and the vaccination against SARS-COV 2.³

The liver is the major organ which is responsible for the production of thrombopoietin. However, decrease in the thrombopoietin level can be associated with decreased liver function.

Therefore, liver function of all the patients should have been evaluated before labeling them as relapsed ITP secondary to post-COVID vaccination.

Some infections like dengue, malaria, and severe fever with thrombocytopenia syndrome (SFTs)⁴ may have resulted in thrombocytopenia. Was any investigation done to rule it out?

In defining patient characteristics, 28 patients received prior treatment with eltrombopag, 2 received romiplostim, and 19 received prior treatment of rituximab. However, no patient having steroid intake is mentioned, as shown in Table 1. As steroid is an ideal non-invasive treatment of ITP, so by specifying steroid-taking patients in the inclusion criteria of Research this could give a better correlation between ITP relapse and steroid intake. Also there seems to be a lack of comparison between the two groups (ie the one who received eltrombopag, romiplostim, and rituximab, and the group who did not receive any treatment) on the effect of the vaccine.

As mentioned in the study, a hyperinflammatory environment in the bone marrow leads to megakaryocytic apoptosis and decrease in platelet concentration. Questions might arise in the reader's mind regarding the pathophysiology. ITP involves dysregulated expansion of specific T-cells subset. The balance between Th-1 and Th-2 subsets regulates the immune response under normal conditions. In contrast, ITP shows an increased Th-1/Th-2 ratio. Also B-cell activating factor (BAFF) may play a pathogenic role in ITP by promoting the survival of CD19(+) and CD8(+) cells, and increasing the apoptosis of platelets and secretion of IFN-gamma.⁵

Disclosure

The authors report no conflicts of interest in this communication.

References

1. Qasim H, Rahhal A, Husain A, et al. Immune thrombocytopenia relapse in patients who received mRNA COVID-19 vaccines. *J Blood Med.* 2023;14:295–302. PMID: 37082002; PMCID: PMC10112532. doi:10.2147/JBM.S396026
2. Feudjo-Tepie MA, Le Roux G, Beach KJ, Bennett D, Robinson NJ. Comorbidities of idiopathic thrombocytopenic purpura: a population-based study. *Adv Hematol.* 2009;2009:963506. PMID: 19960044; PMCID: PMC2778146. doi:10.1155/2009/963506
3. Nunez-Castilla J, Stebliankin V, Baral P, et al. Potential autoimmunity resulting from molecular mimicry between SARS-CoV-2 spike and human proteins. *Viruses.* 2022;14(7):1415. PMID: 35891400; PMCID: PMC9318917. doi:10.3390/v14071415
4. Zhan L, Huang K, Xia W, et al. The diagnosis of severe fever with thrombocytopenia syndrome using metagenomic next-generation sequencing: case report and literature review. *Infect Drug Resist.* 2022;15:83–89. PMID: 35046673; PMCID: PMC8760998. doi:10.2147/IDR.S345991
5. Zhu XJ, Shi Y, Peng J, et al. The effects of BAFF and BAFF-R-Fc fusion protein in immune thrombocytopenia. *Blood.* 2009;114(26):5362–5367. PMID: 19794139. doi:10.1182/blood-2009-05-217513

Dove Medical Press encourages responsible, free and frank academic debate. The content of the Journal of Blood Medicine 'letters to the editor' section does not necessarily represent the views of Dove Medical Press, its officers, agents, employees, related entities or the Journal of Blood Medicine editors. While all reasonable steps have been taken to confirm the content of each letter, Dove Medical Press accepts no liability in respect of the content of any letter, nor is it responsible for the content and accuracy of any letter to the editor.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>

<https://doi.org/10.2147/JBM.S419849>



Switching from Sucrose-Formulated rFVIII to Octocog Alfa (BAY 81-8973) Prophylaxis Improves Bleed Outcomes in the LEOPOLD Clinical Trials

Gili Kenet, Thomas Moulton, Brian M Wicklund, Sanjay P Ahuja, Miguel Escobar & Johnny Mahlangu

To cite this article: Gili Kenet, Thomas Moulton, Brian M Wicklund, Sanjay P Ahuja, Miguel Escobar & Johnny Mahlangu (2023) Switching from Sucrose-Formulated rFVIII to Octocog Alfa (BAY 81-8973) Prophylaxis Improves Bleed Outcomes in the LEOPOLD Clinical Trials, Journal of Blood Medicine, , 379-388, DOI: [10.2147/JBM.S405624](https://doi.org/10.2147/JBM.S405624)

To link to this article: <https://doi.org/10.2147/JBM.S405624>



© 2023 Kenet et al.



Published online: 07 Jun 2023.



Submit your article to this journal



Article views: 195



View related articles



View Crossmark data

Switching from Sucrose-Formulated rFVIII to Octocog Alfa (BAY 81-8973) Prophylaxis Improves Bleed Outcomes in the LEOPOLD Clinical Trials

Gili Kenet^{1,2}, Thomas Moulton³, Brian M Wicklund⁴, Sanjay P Ahuja⁵, Miguel Escobar⁶, Johnny Mahangu⁷

¹National Hemophilia Center, Sheba Medical Center, Tel HaShomer, Israel; ²The Amalia Biron Thrombosis Research Institute, Tel Aviv University, Tel Aviv, Israel; ³Bayer, Whippany, NJ, USA; ⁴Children's Mercy-Kansas City, Kansas City, MO, USA; ⁵Rainbow Babies & Children's Hospital, Cleveland, OH, USA; ⁶University of Texas Health Science Center, Houston, TX, USA; ⁷Hemophilia Comprehensive Care Center, Faculty of Health Sciences, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand, and National Health Laboratory Service, Johannesburg, South Africa

Correspondence: Gili Kenet, Amalia Biron Thrombosis Research Institute, Tel Aviv University, Tel HaShomer, 52621, Israel, Tel +972-3-5307356, Fax +972-3-5351806, Email Gili.kenet@sheba.health.gov.il

Introduction: Previous clinical trials established the efficacy and safety of sucrose-formulated recombinant factor (F) VIII (rFVIII-FS/Kogenate FS®/Helixate FS®) and octocog alfa (BAY 81-8973/Kovaltry®; LEOPOLD trials).

Aim: To report the results of a post hoc subgroup analysis assessing efficacy and safety outcomes in patients with hemophilia A who were receiving rFVIII-FS prior to enrolling into the LEOPOLD I Part B and LEOPOLD Kids Part A clinical trials and switching to octocog alfa.

Methods: LEOPOLD I Part B (NCT01029340) and LEOPOLD Kids Part A (NCT01311648) were octocog alfa Phase 3, multinational, open-label studies in patients with severe hemophilia A aged 12–65 years and ≤12 years, respectively. Annualized bleeding rate (ABR) was the efficacy endpoint for both studies. Safety endpoints included adverse events (AEs) and development of FVIII inhibitors.

Results: Of the 113 patients in both LEOPOLD trials, 40 (35.4%) patients received rFVIII-FS prophylaxis pre-study and had data available for pre-study total ABR. In LEOPOLD I Part B (n = 22, 35.5%), median (Q1; Q3) total ABR decreased from 2.5 (0.0; 9.0) pre-study to 1.0 (0.0; 6.8), and from 1.0 (0.0; 6.0) pre-study to 0.0 (0.0; 6.02) in LEOPOLD Kids Part A (n = 18, 35.3%). Octocog alfa was well tolerated, and no patients had drug-related serious AEs or inhibitors.

Conclusion: Treatment with octocog alfa prophylaxis appeared to have a favorable risk–benefit profile compared with rFVIII-FS and thus could be an effective and improved alternative strategy for individualized treatment for children, adolescent and adult patients with severe hemophilia A currently on rFVIII-FS treatment.

Keywords: FVIII, hemophilia A, prophylaxis, recombinant proteins, octocog alfa

Introduction

Hemophilia A is caused by impaired thrombin generation due to an absence or deficiency of factor VIII (FVIII).¹ Regular replacement therapy with FVIII products, known as prophylaxis, is considered the standard of care.¹ For patients receiving their initial therapy, factors including efficacy, safety, cost and supply/availability might determine the choice of FVIII product.^{2,3} There are multiple drivers to switch treatment products in patients with hemophilia A, including national contracting, access to products and patient preference. The key reasons for switching to an alternative treatment are the clinical benefits for patients, including improved efficacy or pharmacokinetic (PK) profile, thus providing an overall better treatment outcome than the current FVIII treatment. As a result of the evolving treatment landscape in hemophilia, it is unusual for adult patients to use the same FVIII concentrate throughout their lives.³ There are, however, both patient and physician barriers to switching and often a reluctance to change products. These barriers include

concerns of perceived possible negative outcomes, such as increased risk of FVIII-inhibitor development and strong patient preference to continue their current therapy, often due to the development of a strong psychological link with the product they use.^{2,3}

Sucrose-formulated recombinant FVIII (rFVIII-FS, Kogenate FS®/Helixate FS®) and octocog alfa (BAY 81–8973, Kovaltry®) are unmodified, full-length, recombinant FVIII proteins, indicated for prophylaxis and treatment of bleeds in children and adults with hemophilia A since 1993 and 2016, respectively.^{4,5} These approvals were based on the results from previous clinical trials demonstrating the efficacy and safety of on-demand and prophylactic treatment with rFVIII-FS (including the SPINART trial) and octocog alfa (LEOPOLD trials) in patients with hemophilia A.^{6–14} While rFVIII-FS and octocog alfa are full-length human rFVIII products that share the same primary amino acid sequence, these products differ in the way they are manufactured. No human or animal proteins are used in the final formulation steps during the manufacturing of rFVIII-FS, unlike its predecessor rFVIII product, although the cell culture medium has human plasma protein in the solution.¹⁵ Octocog alfa, on the other hand, contains no human or animal-derived raw materials added to the cell culture, purification or formulation processes.^{15–17} In addition, improved manufacturing methodologies including viral filtration, enhanced viability of the expression cell line using heat shock protein 70 and ultra-fast membrane-based capture technology resulted in a rFVIII product with high and consistent purity. Moreover, octocog alfa has key structural changes such as increased branching and sialylation on N-terminal glycan groups compared with its predecessors, known to be critical to the half-life of some mammalian proteins.^{15–17}

PK assessments based on both one-stage clotting and chromogenic assays indicated a favorable PK profile for octocog alfa with a higher area under the curve and half-life, and a markedly lower clearance compared with rFVIII-FS.¹⁵ The observed favorable PK profile for octocog alfa compared with rFVIII-FS was noted across all age groups, particularly in the pediatric age group, and a high proportion of adults and adolescent patients achieved sustained FVIII levels $\geq 1\%$.^{11,12,15} Octocog alfa also demonstrated a superior PK profile compared with antihemophilic factor (recombinant) plasma/albumin-free method (rAHF-PFM/Advate®) in a randomized cross-over head-to-head comparison.¹⁸ These PK studies provided rationale for exploring the efficacy of switching to octocog alfa in previously FVIII-treated patients.

The efficacy and safety of octocog alfa in previously treated patients were assessed in the phase 3 LEOPOLD I Part B and LEOPOLD Kids Part A trials.^{11,12} Upon study entry, patients switched from their previous FVIII treatment to octocog alfa. Here, we report the results of a post hoc subgroup analysis that aimed to assess the efficacy and safety outcomes of patients with hemophilia A who were receiving rFVIII-FS prior to enrolling into the LEOPOLD I Part B and LEOPOLD Kids Part A clinical trials and subsequently switched to octocog alfa upon study entry.

Materials and Methods

Patients and Study Design

The eligibility criteria and study designs of the LEOPOLD I Part B and LEOPOLD Kids Part A trials have been described previously.^{11,12} Briefly, LEOPOLD I Part B and LEOPOLD Kids Part A were phase 3, multinational, open-label studies in patients with severe hemophilia A (ClinicalTrials.gov identifiers: NCT01029340 and NCT01311648, respectively).

In LEOPOLD I Part B, males aged 12–65 years with severe hemophilia A (FVIII $<1\%$) previously treated with any FVIII product for ≥ 150 exposure days (EDs) were eligible for inclusion.¹¹ In LEOPOLD Kids Part A, patients aged ≤ 12 years with severe hemophilia A (FVIII $<1\%$) previously treated with any FVIII product for ≥ 50 EDs were eligible for inclusion.¹² In both the LEOPOLD trials, key exclusion criteria included the presence or history of FVIII inhibitors, diagnosis of any bleeding disorder other than hemophilia A, platelet count $<100,000/\text{mm}^3$, abnormal renal function or clinically relevant liver disease.

In LEOPOLD I Part B, patients received octocog alfa prophylaxis as intravenous administrations of 20–50 IU/kg twice or three times weekly, with the nominal dose and dosing frequency maintained throughout the study (Figure 1). Patients were randomized 1:1 to receive octocog alfa with potency of the dose determined by the chromogenic substrate assay per European Pharmacopoeia (CS/EP) or adjusted by a predefined factor to mimic results obtained with the one-stage assay (CS/ADJ) for a 6-month treatment period. Patients then crossed over to the alternate potency for another

6-month treatment period. The primary efficacy endpoint for LEOPOLD I Part B was annualized bleeding rate (ABR; defined as spontaneous and trauma-related bleeds, untreated bleeds and unspecified bleeds) in each 6-month potency period and over the whole year of treatment, independent of potency.¹¹

In LEOPOLD Kids Part A, patients received 20–50 IU/kg octocog alfa prophylaxis for ≥ 2 times weekly as determined by the investigator in conjunction with the patient/caregiver (Figure 1). The dose of octocog alfa infusions could be changed at any time. The primary efficacy endpoint was ABR occurring within 48 hours after prophylaxis infusion.

Bleeding events and administered infusions were recorded by patients and parents/caregivers using an electronic patient diary, during the LEOPOLD I Part B and LEOPOLD Kids Part A trials, respectively. ABRs were calculated for all bleeds (sum of spontaneous bleeds, trauma bleeds, untreated bleeds and unspecified bleeds) as well as joint, spontaneous, and trauma-related bleeds throughout each study. Pre-study ABRs were calculated using self-reported data based on patient/carer recall of the bleeding events at the baseline visit. Throughout the study, patients were closely monitored at each study visit for the incidence of adverse events (AEs) and also immunogenicity, including FVIII inhibitor development. Inhibitor development was defined as a Nijmegen-modified Bethesda assay measured titre of ≥ 0.6 BU and confirmed in a second plasma sample.

Both studies were approved by the independent ethics committee or institutional review board responsible for each site and were carried out in compliance with the protocol, the principles of the Declaration of Helsinki, and the International Conference on Harmonization guidelines for Good Clinical Practice. All patients or their guardians provided written informed consent. As this is a post hoc subgroup analysis of previously reported studies that had each received ethical approval, additional approval was not sought.

Statistical Analyses

Statistical analysis was performed using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA). Summary statistics were calculated for continuous data, and frequencies were calculated for categorical data. Analyses performed in this study were descriptive. Further details on statistical analyses performed in the main studies are described in previous publications.^{11,12}

Results

Patients

Out of a total of 113 patients who were treated with octocog alfa in LEOPOLD I Part B and LEOPOLD Kids Part A, a total of 40 (35.4%) patients previously received rFVIII-FS prophylaxis treatment prior to enrollment into the respective

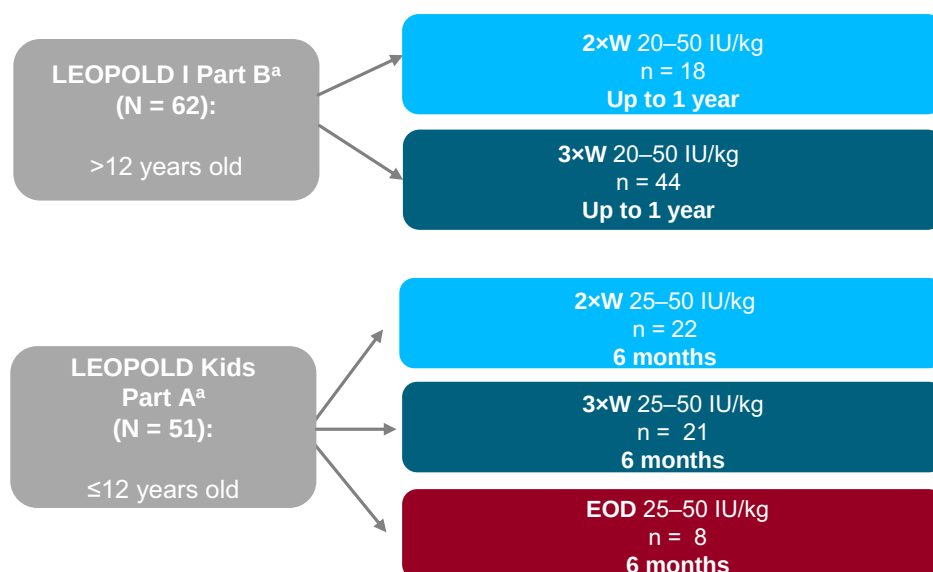


Figure 1 Patient disposition in the LEOPOLD trials. ^aDosing regimens for both studies were assigned by the investigator.

Abbreviations: 2xW, twice weekly; 3xW, three times weekly, EOD, every other day.

LEOPOLD trials and were included in this analysis. Pre-study total ABR data were available for all 40 patients. Disease characteristics and patient demographics at baseline can be found in Table 1. In LEOPOLD I Part B, 22 (35.5%) patients were previously treated with rFVIII-FS prophylaxis and had a median age of 27.0 years; in LEOPOLD Kids Part A, 18 (35.3%) patients were previously treated with rFVIII-FS prophylaxis and had a median age of 5.0 years (Table 1). In general, these switch cohorts had similar patient demographics to the overall study population (Table 1), and any differences did not affect the final analysis.

The majority of patients maintained the same dosing frequency compared with rFVIII-FS in both the LEOPOLD trials (Figure 2). Most patients were receiving prophylaxis three times weekly during the pre-study period and following switching from rFVIII-FS to octocog alfa. The majority of patients in the LEOPOLD I Part A trial switched to a higher dose of octocog alfa compared with rFVIII-FS, at their physician's discretion. The median (range) prescribed dose of previous rFVIII-FS prophylaxis treatment was 28.4 (10.0–44.9) IU/kg. The corresponding value for octocog alfa prophylaxis was 30 (20.0–40.0) IU/kg.

Bleeding Outcomes

Of patients who switched from rFVIII-FS prophylaxis, 31.8% and 55.6% of prophylaxis patients had zero bleeds in LEOPOLD I Part B and LEOPOLD Kids Part A, respectively. Zero bleed data prior to entry to LEOPOLD I and LEOPOLD Kids studies were not available. In LEOPOLD I Part B (n = 22), median (quartile, Q1; Q3) total ABR decreased from 2.5 (0.0; 9.0) in the 12 months of rFVIII-FS treatment prior to study entry, to 1.0 (0.0; 6.8) (Figure 3) – a reduction in total median ABR of 60%. In LEOPOLD I Part B, median (Q1; Q3) trauma-related ABR was 0.0 (0.0; 1.0).

In LEOPOLD Kids Part A (n = 18), median (Q1; Q3) total ABR decreased from 1.0 (0.0; 6.0) in the 12 months of rFVIII-FS treatment prior to study entry, to 0.0 (0.0; 6.02); median (Q1; Q3) total ABR within 48 hours after octocog alfa prophylaxis was 0.0 (0.0; 5.65). Trauma-related median (Q1; Q3) ABR for all patients (n = 18) was 0.0 (0.0; 2.0). In the 0–<6 years cohort, median (Q1; Q3) total ABR in the pre-study was 1.0 (1.0; 12.0), whereas upon switching to octocog alfa, median (Q1; Q3) total ABR within 48 hours after prophylaxis was 0.0 (0.0; 5.65) (Figure 3). Trauma-related median (Q1; Q3) ABR in this cohort was 0.0 (0.0; 5.65), whereas this was zero within 48 hours after prophylaxis. In the 6–12

Table 1 Baseline Demographics and Disease Characteristics

	LEOPOLD I Part B (>12 Years) ^a		LEOPOLD Kids Part A (≤12 Years)	
	Previous rFVIII-FS PPX	Overall Study Population	Previous rFVIII-FS PPX	Overall Study Population
Patients, n (%)	22 (35.5) ^b	62 (100) ^c	18 (35.3)	51 (100) ^c
Age, years, median (range)	27.0 (14–61)	30.0 (12–61)	5.0 (2–11)	6.0 (1–11)
12–<18 years, n (%)	5 (22.7)	10 (16.1)	–	–
Patients with target joints, n (%)	12 (54.5)	44 (71.0)	4 (22.2)	14 (27.5)
0	10 (45.5)	18 (29.0)	14 (77.8)	37 (72.5)
I	6 (27.3)	20 (32.3)	4 (22.2)	9 (17.6)
≥2	6 (27.3)	24 (38.7)	–	5 (9.8)
Baseline ABR, median (range) ^d	2.5 (0–24)	5.5 (0–55)	1.0 (0–20)	4.0 (0–55)

Notes: ^aDifferences in the demographics of the LEOPOLD I cohort may be due to the increased number of OD patients in the total group vs the rFVIII-FS group; they have no effect on the final results. ^bITT population. ^cSafety population. ^dBased on patient/carer recall of bleeding events during the 12 months prior to study entry.

Abbreviations: ABR, annualized bleeding rate; ITT, intention-to-treat; OD, on-demand; PPX, prophylaxis; rFVIII-FS, sucrose-formulated recombinant factor VIII.

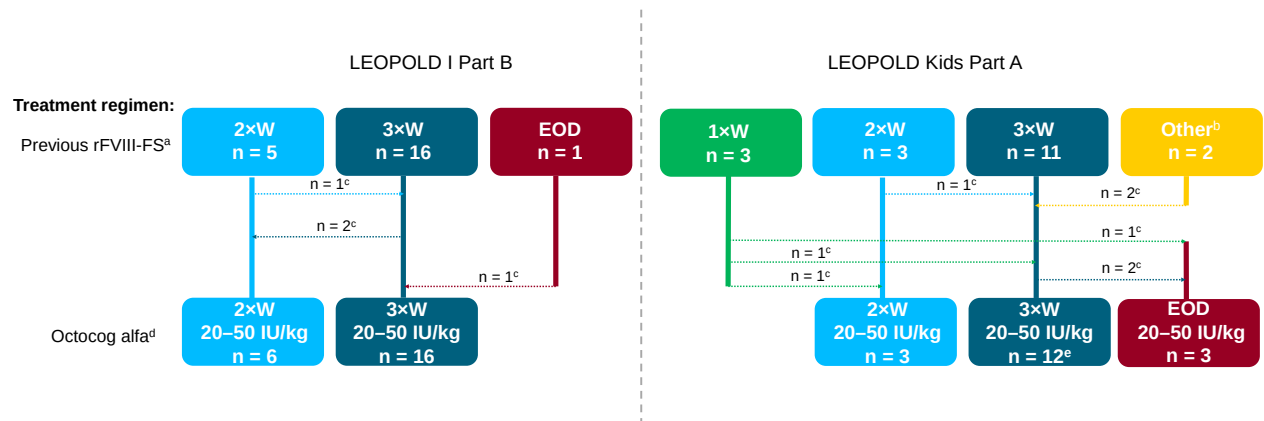


Figure 2 Treatment regimen switching from rFVIII-FS to octocog alfa. ^aData unavailable for previous rFVIII-FS dose; recommended dose for rFVIII-FS prophylaxis is 25 IU/kg 3xW (adults) or 25 IU/kg EOD (children). ^bThese two patients had an estimated mean dosing frequency prior to the study of 0.39 doses per week and 4.02 doses per week, respectively. ^cDotted lines represent the overall shift in prophylaxis regimen between pre-study and the start of the respective LEOPOLD studies; bold lines indicate the overall number of patients in each regimen at the start of each LEOPOLD study in relation to the respective previous rFVIII-FS regimens. ^dPatients were assigned to respective octocog alfa dosing regimens at the investigator's discretion; the majority of patients remained on their previous dosing interval. ^eOne patient was represented in more than one previous treatment category.

Abbreviations: 1xW, once weekly; 2xW, twice weekly; 3xW, three times weekly; EOD, every other day; rFVIII-FS, sucrose-formulated recombinant factor VIII.

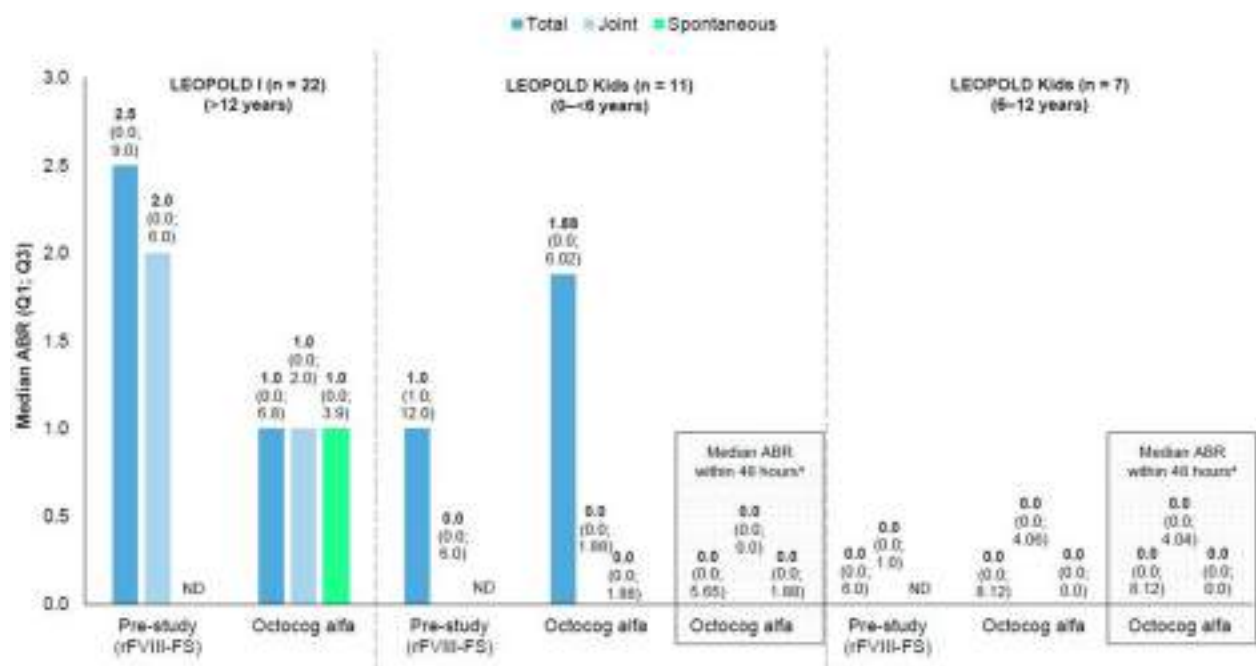


Figure 3 ABR outcomes by age in patients previously treated with rFVIII-FS who switched to octocog alfa prophylaxis in the LEOPOLD studies. ^aThe primary efficacy endpoint in the LEOPOLD Kids study was ABR occurring within 48 hours after prophylaxis infusion, selected because of variable treatment intervals in children, for whom a low infusion frequency could also be related to venous access problems.

Abbreviations: ABR, annualized bleeding rate; ND, no data; Q, quartile; rFVIII-FS, sucrose-formulated recombinant factor VIII.

years cohort, median total ABRs in the pre-study and the LEOPOLD Kids Part A study were zero; trauma-related median ABR was also zero in the study. The median total ABR within 48 hours after prophylaxis in this cohort was also zero. Median joint ABRs in the pre-study and the LEOPOLD Kids Part A study were zero for both 0–<6 and 6–12 years cohorts. Joint median ABRs within 48 hours after prophylaxis were also zero for both 0–<6 years and 6–12 years cohorts.

While pre-study spontaneous ABRs were not available for the 0–<6 and 6–12 years cohorts, spontaneous median ABRs were zero for both cohorts treated with octocog alfa. Similarly, spontaneous median ABRs within 48 hours after prophylaxis were zero for both 0–<6 and 6–12 years cohorts.

Octocog Alfa Utilization

For those who switched from rFVIII-FS to octocog alfa prophylaxis treatment, median (Q1; Q3) FVIII utilization and dose per infusion in LEOPOLD I Part B were 5404.7 (3785.1; 5648.6) IU/kg/year and 35.4 (32.1; 37.2) IU/kg, respectively (Table 2). The corresponding median (Q1; Q3) values for the switch cohort in LEOPOLD Kids Part A were 5810.0 (4359.4; 6358.6) IU/kg/year and 35.2 (28.5; 40.1) IU/kg, respectively (Table 2). rFVIII-FS utilization data prior to entry to LEOPOLD I and LEOPOLD Kids studies were not available.

Safety

Overall, the safety profile of those patients who switched from rFVIII-FS to octocog alfa prophylaxis was similar to that of the overall population in the LEOPOLD I Part B and LEOPOLD Kids Part A trials. Of those patients previously treated with rFVIII-FS, AEs were reported in 77.3% (n = 17) and 61.1% (n = 11) of patients in LEOPOLD I Part B and LEOPOLD Kids Part A, respectively (Table 3). No serious or severe AEs were reported (Table 3). There were no discontinuations in LEOPOLD I Part B; one patient discontinued in the LEOPOLD Kids Part A trial (Table 3). No FVIII inhibitors developed in any patients in either study. No deaths or thrombotic events, including thrombotic microangiopathy, were reported.

Table 2 Octocog Alfa Prophylaxis Utilization^a

	LEOPOLD I (Part B) n = 22	LEOPOLD Kids (Part A)		
		0–<6 Years, n = 11	6–12 Years, n = 7	Total, n = 18
Median dose per year, IU/kg/year (Q1; Q3)	5404.7 (3785.1; 5648.6)	5740.3 (3786.8; 6428.8)	5879.8 (4359.4; 6358.6)	5810.0 (4359.4; 6358.6)
Median dose per infusion, IU/kg/inf (Q1; Q3)	35.4 (32.1; 37.2)	34.0 (28.5; 39.0)	37.6 (25.5; 45.0)	35.2 (28.5; 40.1)

Notes: ^aData for rFVIII-FS prophylaxis utilization were not available.

Abbreviations: Q, quartile; rFVIII-FS, sucrose-formulated recombinant factor VIII.

Table 3 TEAEs Reported in Patients During the LEOPOLD Studies Previously Treated with rFVIII-FS

TEAEs	LEOPOLD I (Part B) n = 22	LEOPOLD Kids (Part A) n = 18
Any AE, n (%)	17 (77.3)	11 (61.1)
Maximum intensity for any AE		
Mild, n (%)	9 (40.9)	8 (44.4)
Moderate, n (%)	8 (36.4)	3 (16.7)
Severe, n (%)	0 (0)	0 (0)
Any study-drug-related AE, n (%)	0 (0)	0 (0)
Discontinuation due to AE or SAE, n (%)	0 (0)	1 (5.6) ^a

Notes: ^aPatient discontinuation was due to central venous catheter–related infection after 6 months of treatment (rated as moderate intensity and not considered study-drug-related).

Abbreviations: AE, adverse event; rFVIII-FS, sucrose-formulated recombinant factor VIII; SAE, serious AE; TEAE, treatment-emergent AE.

Discussion

The results demonstrate that octocog alfa prophylaxis provides improved bleed protection, defined by a reduction in ABR, compared with similar dosing regimens of rFVIII-FS prophylaxis.

In LEOPOLD I Part B and LEOPOLD Kids Part A, patients who switched from rFVIII-FS to octocog alfa had reduced total ABRs compared with pre-study. In the LEOPOLD Kids Part A study, an increased total ABR was observed in the 0–<6 years cohort when these patients switched from rFVIII-FS to octocog alfa. This observed increase in ABR could be due to carer recall bias on pre-study bleeding events, leading to a lower or less accurate pre-study ABR. It is also plausible that increased trauma-related complications observed in younger children compared with older children and adults could have resulted in the observed increase in total ABR.^{19–21} Of note, some patients in the 0–<6 years cohort would have been in their more active developmental stage (eg, toddlers) during the study and hence incurred more injuries compared with their relatively less active developmental stage in the pre-study.^{20,22} Nevertheless, a reduction in total ABR within 48 hours after prophylaxis (primary efficacy outcome in LEOPOLD Kids Part A) was observed in this cohort after switching to octocog alfa, compared with pre-study. Whilst the primary efficacy endpoint in both LEOPOLD I Part B and LEOPOLD Kids Part A was ABR, this was reported differently. The 48-hour endpoint was selected in LEOPOLD Kids Part A to account for the variable treatment intervals in children, for whom a low infusion frequency could also be related to venous access problems. This post hoc subgroup analysis did not aim to compare results between the LEOPOLD studies.

Joint ABRs were lower in the LEOPOLD I Part B switch cohort, compared with pre-study joint ABRs. Although spontaneous ABRs in LEOPOLD I Part B, and joint and spontaneous ABRs in LEOPOLD Kids Part A were low, a comparison of efficacy is not possible due to unavailability of corresponding pre-study data for these patients. The efficacy results of this subgroup analysis complement those of the LEOPOLD I and LEOPOLD Kids primary analyses.^{11,12}

Most patients in either switch cohort maintained their dosing frequency while switching from rFVIII-FS to octocog alfa in the LEOPOLD trials. Better bleed protection with octocog alfa treatment, compared with rFVIII-FS, was also attained using dosing within the approved recommended range for adults and children (20–40 IU/kg and 25–50 IU/kg, respectively), as evidenced from the median dose per infusion received by the switch cohorts in both studies (35.4 IU/kg in LEOPOLD I Part B and 35.2 IU/kg in LEOPOLD Kids Part A). The overall improved efficacy outcomes with octocog alfa observed in this subgroup analysis indicate that most patients currently receiving rFVIII-FS could switch to octocog alfa for better bleed protection. As supported by the dosing recommendations, these data suggest that octocog alfa can also be used with fewer infusions compared with rFVIII-FS.^{4,5}

Octocog alfa was well tolerated in the switch cohorts, with a safety profile similar to that of the overall population in both LEOPOLD studies.^{11,12} There were no study-drug-related AEs or serious AEs in either of the switch cohorts. No patients discontinued treatment in LEOPOLD I Part B; one patient in LEOPOLD Kids Part A discontinued treatment due to non-study-drug-related central venous catheter-related infection. No immunogenicity concerns were observed, with no patients developing FVIII inhibitors. Switching from rFVIII-FS to octocog alfa prophylaxis did not result in FVIII inhibitor development — a major concern among physicians and patients who are reluctant in switching from their current FVIII therapy.^{2,3} The most recent guidelines from the World Federation of Hemophilia state that current evidence indicates no increased risk of inhibitor development upon product switching.¹ The better efficacy profile observed with octocog alfa treatment over rFVIII-FS in the LEOPOLD switch cohorts could potentially be attributed to the favorable PK profile of octocog alfa as demonstrated in the LEOPOLD PK assessments.^{11,15}

This analysis has a few limitations. First, this is a post hoc study on a limited number of patients with no intra-individual analysis performed. Second, the pre-study data were collected retrospectively based on patient-reported data on bleeds. Pre-study data collection on bleeds relied on a patient's recall of bleeding events, with data unavailable for a comparison of joint and/or spontaneous ABRs. However, the LEOPOLD studies were prospective in nature, with data on bleeds while on octocog alfa treatment being noted in patient diaries. Third, some of the patients in the switch cohorts were receiving rFVIII-FS below the recommended dose of 25 IU/kg.⁴ As this was a global study, in some countries, patients may not have been able to dose according to the prescribing information and thus the data may not be

representative of all countries.⁴ Hence, these patients may have had insufficient prophylaxis prior to study entry. Fourth, the recommended dose of 25 IU/kg for rFVIII-FS is less than the recommended dose for octocog alfa (20–40 IU/kg). Thus, using a higher dose of octocog alfa on the same dosing regimen as rFVIII-FS could also improve ABR. Consequently, the extent of differences observed in ABRs in these patients could be an overestimation. Finally, it is plausible that the observed improvement in ABR could be due to a potential improvement in adherence to treatment plan associated with clinical trials.²³ Nevertheless, based on this post hoc analysis and previous PK comparisons, treatment with octocog alfa prophylaxis appears to have a favorable risk–benefit profile compared with rFVIII-FS. TAURUS, a recent observational study investigating real-world prophylaxis, found that patients switching from previous FVIII treatment to octocog alfa had stable annualised bleeding rates, treatment satisfaction and adherence over a period of 1–2 years with no new or unexpected safety concerns; indicating that for patients with moderate-to-severe haemophilia A, octocog alfa remains a useful treatment option.²⁴

Conclusion

The results of this post hoc subgroup analysis indicate that octocog alfa prophylaxis could be an effective and improved, alternative strategy for individualized treatment for children, adolescent and adult patients with severe hemophilia A, currently on rFVIII-FS treatment.

Abbreviations

ABR, annualized bleeding rate; AE, adverse event; ED, exposure day; FVIII, factor VIII; PK, pharmacokinetic; rFVIII-FS, sucrose-formulated recombinant FVIII.

Full List of Approving IRBs (TUN)

De Videnskabsetiske Komitéer for Region Midtjylland (35001),
 Medizinische Einrichtungen der Universität Bonn Ethikkommission an der Medizinischen Fakultät (10001),
 Chaim Sheba Medical Center Helsinki Committee Tel-Hashomer (39001),
 Comitato Etico a.o. Pugliese-Ciaccio (22002),
 Comitato Etico Per Le Attività Biomediche Carlo Romano Università Studi Federico II (22004),
 Comitato Etico Per La Sperimentazione Clinica Della Provincia Di Vicenza (22003),
 Comitato Di Etica Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (22001),
 Komisja Bioetyczna przy Inst. Hematologii i Transfuzjologii (18001, 18002),
 University of Pretoria, Prinshof Campus (37002),
 University of Witwatersrand WITS Human Research Ethics Committee (Medical) (37001),
 Ciutat Sanitària i Universitària de la Vall d'Hebron (24001),
 Comité Ético d'Investigació Clínica, Complejo Hospitalario Universitario A Coruña
 Servicio Galego de Saude – SERGAS Comité Ético de Investigación Clínica de Galicia (24002),
 Hospital Central de Asturias Secretaría del Comité Ético de Investigación Clínica (24006),
 Hospital Universitari i Politècnic La Fe Comité Etico de Investigación Clínica / Fundación para la investigación (24003),
 Regionala Etikprövningsnämnden i Göteborg (34003),
 Ege Üniversitesi Tıp Fakültesi Ege Üniversitesi Tıp Fakültesi İzmir 1 nolu Klinik Araştırmalar Etik Kurulu (47001, 47002, 47003),
 East Midlands - Derby Ethics Committee,
 Children's Hospital Boston Committee on Clinical Investigation (14002),
 Children's Mercy Hospital Pediatric Institutional Review Board (14009),
 St. Joseph's Hospital Tampa Florida (14001),
 University Hospitals Case Medical Center IRB for Human Investigation (14005),
 University of California – Davis IRB Administration (14006).

Data Sharing Statement

Availability of the data underlying this publication will be determined later according to Bayer's commitment to the EFPIA/PhRMA "Principles for responsible clinical trial data sharing". This pertains to scope, time point and process of data access. As such, Bayer commits to sharing upon request from qualified scientific and medical researchers, patient-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in patients for medicines and indications approved in the United States (US) and European Union (EU) as necessary for conducting legitimate research. This applies to data on new medicines and indications that have been approved by the EU and US regulatory agencies on or after January 01, 2014. Interested researchers can use www.clinicalstudydatarequest.com to request access to anonymized patient-level data and supporting documents from clinical studies to conduct further research that can help advance medical science or improve patient care. Information on the Bayer criteria for listing studies and other relevant information is provided in the Study sponsors section of the portal. Data access will be granted to anonymized patient-level data, protocols and clinical study reports after approval by an independent scientific review panel. Bayer is not involved in the decisions made by the independent review panel. Bayer will take all necessary measures to ensure that patient privacy is safeguarded.

Acknowledgments

The authors thank Graeme Baldwin of Darwin Healthcare Communications (Oxford, England) for providing medical writing support, which was fully funded by Bayer, in accordance with Good Publication Practice (GPP3) guidelines.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study and the LEOPOLD studies were funded by Bayer.

Disclosure

G. Kenet: is a member of the Board of Directors/advisory committees and has received consultancy fees from ASC therapeutics, Roche, Sobi, Uniquore, Bayer, Pfizer, BioMarin, Takeda, Roche, Novo Nordisk, Sanofi, PI Healthcare and CSL Behring; has received research funding from BSF, Opko Biologics, Bayer, Pfizer, Roche, Alnylam (Sanofi) and Shire; has received honoraria for participation in speakers' bureaus from Bayer, BPL, CSL, Roche, Sanofi-Genzyme, Sobi, Spark, Uniquore, Pfizer, Takeda, BioMarin and Novo Nordisk. T. Moulton: is a Bayer employee. B. M. Wicklund: has received consultancy fees from Bayer, Genentech, Novo Nordisk and Shire. S. P. Ahuja: has received consultancy fees from Genentech, Sanofi Genzyme, CSL Behring and XaTek, Inc.; has received patent royalties and research funding from XaTek, Inc. He also has a patent with royalties for ClotChip and a patent for TraumaChek issued to US Patent office. M. Escobar is a member of the advisory boards and/or has received consultancy fees from Biomarin, Novo Nordisk, CSL Behring, Genentech/Roche, Sanofi, Takeda, Pfizer, Bayer, Kedrion, Hemobiologics/LFB, National Hemophilia Foundation, UniQure. J. Mahlangu is a member of the scientific advisory committee of Amgen, Bayer, Baxalta, Biogen, Biotest, CSL Behring, Catalyst Biosciences, Novo Nordisk, Roche and Spark; has received research grants from Bayer, Biogen, BioMarin, CSL Behring, Novartis, Sanofi, Spark, Takeda, Novo Nordisk, Sobi, Roche, Uniqure; has received honoraria for participation in speakers' bureaus from Alnylam, Bayer, Biogen, Biotest, ISTH, Novo Nordisk, Pfizer, Roche, Sobi, Shire, and World Federation of Hemophilia. The authors report no other conflicts of interest in this work.

References

1. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia, 3rd edition. *Haemophilia*. 2020;26(Suppl 6):1–158. doi:10.1111/hae.14046

2. Santagostino E, Auerswald G, Benson G, et al. Switching treatments in haemophilia: is there a risk of inhibitor development? *Eur J Haematol*. 2015;94(4):284–289. doi:10.1111/ejh.12433
3. Iorio A, Puccetti P, Makris M. Clotting factor concentrate switching and inhibitor development in hemophilia A. *Blood*. 2012;120(4):720–727. doi:10.1182/blood-2012-03-378927
4. Kogenate-w-vial-adaptor prescribing information. Bayer; 2019.
5. Kovaltry. prescribing information. Bayer; 2016.
6. Kreuz W, Gill JC, Rothschild C, et al. Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A: results of an international clinical investigation. *Thromb Haemost*. 2005;93(3):457–467. doi:10.1160/TH03-10-0643
7. Collins P, Faradji A, Morfini M, Enriquez MM, Schwartz L. Efficacy and safety of secondary prophylactic vs. on-demand sucrose-formulated recombinant factor VIII treatment in adults with severe hemophilia A: results from a 13-month crossover study. *J Thromb Haemost*. 2010;8(1):83–89. doi:10.1111/j.1538-7836.2009.03650.x
8. Abshire TC, Brackmann HH, Scharrer I, et al. Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy--International Kogenate-FS Study Group. *Thromb Haemost*. 2000;83(6):811–816. doi:10.1055/s-0037-1613925
9. Manco-Johnson MJ, Kempton CL, Reding MT, et al. Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART). *J Thromb Haemost*. 2013;11(6):1119–1127. doi:10.1111/jth.12202
10. Kavakli K, Yang R, Rusen L, Beckmann H, Tseneklidou-Stoeter D, Maas Enriquez M. Prophylaxis vs. on-demand treatment with BAY 81-8973, a full-length plasma protein-free recombinant factor VIII product: results from a randomized trial (LEOPOLD II). *J Thromb Haemost*. 2015;13(3):360–369. doi:10.1111/jth.12828
11. Saxena K, Lalezari S, Oldenburg J, et al. Efficacy and safety of BAY 81-8973, a full-length recombinant factor VIII: results from the LEOPOLD I trial. *Haemophilia*. 2016;22(5):706–712. doi:10.1111/hae.12952
12. Ljung R, Kenet G, Mancuso ME, et al. BAY 81-8973 safety and efficacy for prophylaxis and treatment of bleeds in previously treated children with severe haemophilia A: results of the LEOPOLD Kids trial. *Haemophilia*. 2016;22(3):354–360. doi:10.1111/hae.12866
13. Oldenburg J, Windyga J, Hampton K, et al. Safety and efficacy of BAY 81-8973 for surgery in previously treated patients with haemophilia A: results of the LEOPOLD clinical trial programme. *Haemophilia*. 2016;22(3):349–353. doi:10.1111/hae.12839
14. Mahlangu J, Lopez Fernandez MF, Santagostino E, et al. BAY 81-8973 demonstrated efficacy, safety and joint status improvement in patients with severe haemophilia A in the LEOPOLD I extension for ≤ 2 years. *Eur J Haematol*. 2020;104(6):594–601. doi:10.1111/ejh.13402
15. Shah A, Delesen H, Garger S, Lalezari S. Pharmacokinetic properties of BAY 81-8973, a full-length recombinant factor VIII. *Haemophilia*. 2015;21(6):766–771. doi:10.1111/hae.12691
16. Ishaque A, Thrift J, Murphy JE, Konstantinov K. Over-expression of Hsp70 in BHK-21 cells engineered to produce recombinant factor VIII promotes resistance to apoptosis and enhances secretion. *Biotechnol Bioeng*. 2007;97(1):144–155. doi:10.1002/bit.21201
17. Garger S, Severs J, Regan L, et al. BAY 81-8973, a full-length recombinant factor VIII: manufacturing processes and product characteristics. *Haemophilia*. 2017;23(2):e67–e78. doi:10.1111/hae.13148
18. Shah A, Solms A, Garmann D, et al. Improved pharmacokinetics with BAY 81-8973 versus antihemophilic factor (recombinant) plasma/albumin-free method: a randomized pharmacokinetic study in patients with severe hemophilia A. *Clin Pharmacokinet*. 2017;56(9):1045–1055. doi:10.1007/s40262-016-0492-2
19. Kulkarni R, Presley RJ, Lusher JM, et al. Complications of haemophilia in babies (first two years of life): a report from the centers for disease control and prevention universal data collection system. *Haemophilia*. 2017;23(2):207–214. doi:10.1111/hae.13081
20. Bradshaw CJ, Bandi AS, Muktar Z, et al. International study of the epidemiology of paediatric trauma: PAPS research study. *World J Surg*. 2018;42(6):1885–1894. doi:10.1007/s00268-017-4396-6
21. Barg AA, Livnat T, Budnik I, et al. Efficacy and monitoring in a paediatric cohort: real-world data. *Br J Haematol*. 2020;191(2):282–290. doi:10.1111/bjh.16964
22. Chaudhary S, Figueroa J, Shaikh S, et al. Pediatric falls ages 0–4: understanding demographics, mechanisms, and injury severities. *Inj Epidemiol*. 2018;5(Suppl 1):7. doi:10.1186/s40621-018-0147-x
23. Mancuso ME, Oldenburg J, Boggio L, et al. High adherence to prophylaxis regimens in haemophilia B patients receiving rIX-FP: evidence from clinical trials and real-world practice. *Haemophilia*. 2020;26(4):637–642. doi:10.1111/hae.14018
24. Santoro C, Fuh B, Le PQ, et al. Efficacy and safety in patients with haemophilia A switching to octocog alfa (BAY 81-8973): final results of the global real-world study, Taurus. *Eur J Haematol*. 2023;110(1):77–87. doi:10.1111/ejh.13876

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Blood Transfusion Complications and Associated Factors Among Blood-Transfused Adult Patients at Debre Markos Comprehensive Specialized Hospital, Ethiopia: A Cross Sectional Study

Yalew Muche, Yemataw Gelaw, Aytenew Atnaf & Zegeye Getaneh

To cite this article: Yalew Muche, Yemataw Gelaw, Aytenew Atnaf & Zegeye Getaneh (2023) Blood Transfusion Complications and Associated Factors Among Blood-Transfused Adult Patients at Debre Markos Comprehensive Specialized Hospital, Ethiopia: A Cross Sectional Study, Journal of Blood Medicine, , 389-398, DOI: [10.2147/JBM.S412002](https://doi.org/10.2147/JBM.S412002)

To link to this article: <https://doi.org/10.2147/JBM.S412002>



© 2023 Muche et al.



Published online: 08 Jun 2023.



Submit your article to this journal



Article views: 1082



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Blood Transfusion Complications and Associated Factors Among Blood-Transfused Adult Patients at Debre Markos Comprehensive Specialized Hospital, Ethiopia: A Cross Sectional Study

Yalew Muche¹, Yemataw Gelaw², Aytenew Atnaf¹, Zegeye Getaneh²

¹Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Debre Markos University, Debre Markos, Ethiopia;

²Department of Hematology & Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Correspondence: Yalew Muche, Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Debre Markos University, P.O. BOX 269, Debre Markos, Ethiopia, Tel +251927615910, Email enathager08@gmail.com

Background: Blood transfusion is the infusion of whole blood or its components into the veins of the patient to improve tissue oxygenation and maintain hemostasis. Besides its clinical use, it can pose a risk of transfusion complications with different factors.

Purpose: The aim of this study was to assess blood transfusion complications, and associated factors among transfused adult patients at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2022.

Materials and Methods: An institution-based cross-sectional study design was conducted on a total of 182 patients from March 20 to June 15, 2022. Patients were enrolled in the study using consecutive sampling method. The socio-demographic and clinical data were collected using a structured questionnaire and data extraction sheet, respectively. About 3 ml of anti-coagulated blood and 30 ml of urine samples were collected to assess transfusion complications. CBC and Coombs test were performed from blood and urinalysis from urine, respectively. Chi-square, Fisher's exact test, and binary logistic regression were done using SPSS version 25. P-values less than 0.05 are declared as statistically significant.

Results: An acute transfusion reaction (ATR) was encountered in 12 (6.6%) patients. It was 4.13, 7.78 and 3.96 times more likely to occur among patients with a previous history of transfusion, abortion, and transfused blood stored for more than 20 days compared to their counterparts, respectively. In addition, the odds of developing ATR increase by 2.07 as the number of transfused blood units increases by 1 unit.

Conclusion: The incidence of acute transfusion reactions was high. During transfusion, clinicians should closely monitor patients who had history of transfusion, abortion, transfused old blood and more than 1 unit.

Keywords: acute transfusion reactions, adults, transfusion outcome

Introduction

Blood transfusion is a common lifesaving therapy in modern medicine for a variety of diseases to improve tissue oxygenation and maintain hemostasis.^{1,2} Blood transfusion can be whole blood, packed red blood cells (PRBCs), platelets (PLT), plasma, or plasma derivatives.³

Blood transfused patient may develop a transfusion reaction (TR), defined as unintended patient outcomes due to blood product transfusion.⁴ The two types of transfusion reactions (TR) are acute transfusion reactions (ATR) and delayed transfusion reactions (DTR). Acute transfusion reactions occur during the transfusion and for up to 24 hr after the transfusion is stopped.^{5,6} These include acute hemolytic transfusion reaction (AHTR), febrile non-hemolytic transfusion reaction (FNHTR), anaphylactic and simple allergic. Transfusion-related acute lung injury (TRALI), and transfusion associated circulatory overload (TACO) are other ATRs.⁴ An ATR is assessed using clinical findings

(physical examinations and vital signs), and laboratory investigation includes ABO and RhD group, re-cross match, complete blood count (CBC), direct anti-globulin test (DAT), and hemoglobinuria.³

In sub-Saharan Africa, whole blood is widely used for treatment and transfusions beyond their indications, and there is a lack of different pre-transfusion testing reagents that trigger TR.⁴ Whole-blood transfusion is also widely used in Ethiopia,^{7–11} with a component preparation rate of 16.5%.¹² Moreover, in high-income countries, the most frequently transfused patient group is over 65 years of age and accounts for up to 76% of all transfusions. In low- and middle-income countries, pregnancy-related complications, childhood malaria, and trauma-related injuries are common indications.^{12–16}

The burden of ATR also varies in different countries. In Europe and other continents, the prevalence is between 0.23% and 3.6%.^{1,4,17,18} The lowest prevalence is reported in the United States of America (USA) (0.23%), while the highest is found in Bangladesh (3.6%).^{19,20} However, the prevalence of ATR ranges from 2.6% to 5.2%^{8,21,22} in some African countries, whereas in Ethiopia it is not well studied.

Transfusion reaction prevalence is affected by different factors including sex and age,¹⁹ pre-hematological abnormalities in CBC results,²³ clinical severity of disease, storage time of transfused blood, and history of transfusion. Furthermore, factors influencing blood transfusion outcome or complications include abortion history, vital signs, transfused blood component, and the number of transfused units.^{8,24} To summarize, there was variation in blood component transfusion and ATR across the world. In Ethiopia, there is no hemovigilance system, and whole blood is the most commonly transfused blood.

Despite the fact that Ethiopia had a transfusion service for many years, there is still an unfilled gap in the evidence on major indications for blood transfusion complication and associated factors of patient outcomes information. As a result, the purpose of this study was to assess blood transfusion complications and associated factors of ATR in adult transfused patients at Debre Markos Comprehensive Specialized Hospital, Ethiopia (DMCSH) in North West Ethiopia.

Materials and Methods

Study Design, Setting and Period

An institutional-based cross-sectional study was conducted from March 20 to June 15, 2022. The study was conducted at DMCSH, Debre Markos, East Gojjam zone, Amhara regional state, Ethiopia. Debre Markos is located 299 km away from Addis Ababa, the capital city of Ethiopia, in the North West direction and 265 km away from Bahir Dar, the Amhara regional city. The city has a longitude of 37.734398, a latitude of 10.3296345, elevation of 2411m/7910feet. According to 2012 census the population of the city is 262,497.²⁵ The hospital gives services to an estimation of 5 million people from the catchment area and as a referral from other general and primary hospitals. The Hospital has one mini blood bank to distribute blood products to different wards. In this hospital, blood is transfused in surgical, Gynecological/Obstetrics, emergency, and inpatient (medical) wards. The hospital gets blood for transfusion from Debre Markos Blood Bank.²⁶ All blood transfused adult patients at DMCSH were the source population.

Population

Blood transfused adult patients who fulfilled the eligibility criteria at DMCSH during the study period were study populations. In this study, patients who took anesthetic drug were excluded from the study.

Sample Size and Sampling Technique

A total of 182 adult patients were enrolled in the study. All adult patients who received blood and met eligibility requirements were enrolled in the study by consecutive manner.

Data Collection Methods

Socio-Demographic and Clinical Data Collection

Socio-demographic data were collected from voluntary participants using a pretested, structured questionnaire. Clinical data were collected through interviews and patient card reviews and extracted onto a data extraction sheet. General

physical and clinical examinations such as body temperature, blood pressure, pulse rate, respiratory rate, and oxygen saturation percentage were measured as baseline data after resting for at least 5 min.

During the transfusion, vital signs were initially recorded at intervals of 15 min, 30 min, and 1 hr during the transfusion. They were then recorded again at 4 hr after the transfusion had ended. Patients were physically evaluated, and their vital signs were monitored at the same time to look for indicators of a TR. The following symptoms were recorded: headache, palpitations, mild dyspnea, urticaria, nausea, tachycardia, restlessness, vomiting, and dyspnea.

After the transfusion, the monitoring was kept up for 24 hr at 4-hr intervals. Data from the patient chart and/or the laboratory registration logbook were also used to record the blood donation date, the blood group of the transfused blood and the quantity of units transfused. Patients were suspected of having ATR, and laboratory tests were performed when one or more vital signs deviated from baseline values and when signs and symptoms were noticed.

Sample Collection and Laboratory Analysis

Sample Collection

Blood and urine samples were collected from each volunteer patient before and after transfusion. Those samples were used to investigate the post-transfusion outcome and ATR for suspected cases. Seventy percent alcohol was used to clean the skin and waited until it dried. About three milliliters (mL) of blood specimens were collected from each patient and added to tri-potassium ethylene di-amine tetraacetic acid (K3-EDTA) test tube. The collected blood sample was checked for proper labeling, hemolysis, and clotting pre-analytically. It was then properly mixed to prevent clotting, and it was used for CBC testing, ABO and Rh regrouping, rechecking-cross-matching, and DAT testing. About 30mL of urine was collected in a clean urine cup container for urine analysis (UA), which is typically used to determine hemoglobinuria levels.

Laboratory Analysis

For each laboratory test, standard operating procedures were strictly followed. Pre-transfusion CBC (Hb, RBC, WBC, and PLT), and UA results were used as a baseline. These data were obtained from either on a patient chart or requested for those who lack it on their chart. The baseline CBC was performed from a specimen collected for cross-match for those who did not have it on their chart and CBC was analyzed before performing cross-match. Pre- and post-transfusion samples were performed with a similar instrument to avoid instrumental variation. For patients suspected of ATR either clinically or using the screening of urine samples, firstly the labels of transfused blood units and the patient's identity were checked. Additionally, the pre-transfusion of patients' samples was regrouped for ABO and Rh blood types. Then, donor blood samples were rechecked for blood groups, cross-matched, on the other side, the recipient's post-transfusion blood sample was tested for CBC, blood grouping, and screened for in vivo sensitization of RBCs (DAT).

CBC analysis of the K₃-EDTA anti-coagulated blood was carried out automatically by a mindray BC-3000 plus (Mindray, China) three differential hematological analyzer machine at DMCSH. The tests were performed within 2 hr of sample collection. The remaining sample was kept for blood type regrouping, cross match rechecking, and DAT when TRs were suspected. Patient blood ABO, and Rh type were re-grouped using commercially prepared antisera (anti-A, anti-B and anti-D). The slide method was applied to determine ABO and Rh blood groups. If a transfusion reaction was suspected, patient plasma and donor red cells were mixed for compatibility test under saline and anti-globulin conditions. The DAT was performed for all study participants who were suspected of ATR. It was tested by mixing washed patient RBCs directly with the anti-globulin serum for the detection of in vivo sensitization of patient RBCs. Urine dipstick was performed for screening of transfusion reaction followed by microscopic examination to rule out hematuria.

Data Analysis and Interpretation

Data were entered, cleaned using Epidata version 3.1, and exported to statistical package for the social sciences (SPSS) version 25 software for analysis. The result was presented in texts, tables, and figures as numbers and percentages. To determine the average distribution of data, the median with interquartile range and mean with standard deviation were calculated for the participants' age and Hb. The strength of the association was assessed by binary logistic regression. Variables with p-values less than or equal to 0.25 from the output of bi-variable were transferred to multivariable binary

logistic regression. Finally, the odds ratio, 95% confidence interval, and p-value were used to express the strength of the association. P-value of <0.05 is declared statistically significant.

Results

Demographic Characteristics of Study Participants

A total of 182 participants were enrolled in the study. Eighty-nine (54.4%) of the total participants were male. Majority of the study participants 110 (60.4%) were in the age range of 18–35 years, while aged 36–45 years were 29 (15.9%) participants. Females had a median (interquartile range) age of 32 (11) years, while males had a median (interquartile range) age of 30 (23) years. Most of the study participants (81.9%) were rural residents.

Clinical Characteristics of the Participants

Of the total study participants, 95 (52.3%) were transfused in the emergency department, followed by surgical department 33 (18.1%), medical ward 30 (16.5%), gynecology/obstetrics 15 (8.2%), and the least transfusion was done in intensive care unit 9 (4.9%). Out of the total number of transfused patients, 95 (52.2%) were stable based on pre-transfusion clinical assessment. Forty-six (25.3%) participants had previous transfusion histories. Of the total female participants, 66 (79.5%) had a history of pregnancy; among females who had pregnancy history, 13 (19.7%) had an abortion history.

One hundred five and 55 patients were transfused by the orders of general practitioners and specialist doctors, respectively. In this study, 350 mL citrate phosphate dextrose adenine-1 (CPDA-1) preserved whole blood was the only blood transfused during the study period. The mean (standard deviation) of the blood storage time was 17.36 (7.74) days. One hundred twenty five (68.7%) participants were transfused with blood that had a storage time of up to 20 days, and 141 (77.5%) were transfused more than 1 unit of blood (Table 1).

Primary Diagnosis of Transfusion

In the current study, acute anemia (57), chronic anemia (95), and hematological abnormalities (17) are the common reasons for whole-blood transfusion (Table 2).

Acute Transfusion Reaction

One hundred eighty-two patients were assessed for ATR. Fifty-four patients who took anesthetic drugs were excluded because it may mask ATR. The overall incidence of ATR was 12 (6.6%) with 95% confidence interval (95% CI: 3.3–10.7%). Among these ATRs, allergic reactions had the highest proportion, followed by FNHTR with a proportion of 50% and 33.33%, respectively. In addition, alloimmunization and TRALI were encountered with equal proportion of 8.33% (Table 3).

Table 1 Clinical Characteristics of Blood Transfused Adult Patients at DMCSH from March 20 to June 15, 2022

Clinical Variables		Frequency	
		N	%
Transfusion ward	Emergency	95	52.2
	Gynecology/ obstetrics	15	8.2
	Medical inpatient	30	16.5
	Intensive care unit	9	4.9
	Surgical	33	18.1
Pre transfusion clinical status	Stable	95	52.2
	Not stable	87	47.8
Age of blood (days)	≤ 20	125	68.7
	>20	57	31.3
Amount of blood transfused (units)	=1	41	22.5
	>1	141	77.5

Table 2 Indications of Blood Transfusion for Adult Patients at DMCSH from March 20 to June 15, 2022

Indication =182	Underline Disease	Frequency	
		N	%
Chronic anemia	GI bleeding	9	4.9
	Infection	40	22.0
	Metabolic disorder	8	4.4
	Organ associated	28	15.4
	Others	10	5.5
Acute anemia	Gynecological and obstetrics cases	13	7.1
	Trauma, other surgical cases	44	24.2
Hematological	Malignancy	8	4.4
	Bi and pan cytopenia	9	4.9
Anemia	Undefined	10	5.5

Notes: Infection; HIV, HBV, malaria, and Tuberculosis. Metabolic disorder; Diabetes Mellitus, hypertension, and malnutrition. Maternal and gynecological related; retained placenta, uterine atony, myoma, gestational thromboplastin disease (GTD), vaginal bleeding, eclampsia, and abortion. Other surgical-related diseases; Peptic ulcer disease (PUD), hyperthyroidism and ascites; Organ-related; chronic kidney disease, chronic liver disease, and acute kidney disease; Hematological malignancy; (Acute Lymphoid Leukemia) ALL, Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML); Hematological Cytopenia; bicytopenia, and pancytopenia. Others include asthma, epistaxis, and gangrene.

Table 3 Acute Transfusion Reaction Proportion Among Blood Transfused Adult Patients at DMCSH from March 20 to June 15, 2022

Transfusion Reaction (n=182)	No=170	Incidence of ATR		Proportion of ATR %
	Yes=12	N	%	
ATR =12 (6.6%)	Allo-immunization	1	0.55	8.34
	Allergic	6	3.30	50.00
	FNHTR	4	2.20	33.33
	TRALI	1	0.55	8.33
	Total	12	6.60	100.00

Abbreviations: FNHTR, febrile non-hemolytic transfusion reaction; TRALI, transfusion related acute lung injury.

Factors Associated with the Development of ATR

The proportion of ATR was higher in females (8.6%), patients who lived in an urban area (18.2%). In the bivariable binary logistic regression analysis, ATR was associated with urban residence (COR = 5.26, 95% CI: 1.58–17.53), transfusion history (COR = 4.67, 95% CI: 1.4–15.52), abortion history (COR = 9.11, 95% CI: 2.31–40.75), storage time (COR = 4.89, 95% CI: 1.41–17.01), and transfused whole blood increased by 1 unit (COR = 2.42, 95% CI: 1.44–4.06).

Transfusion history (AOR = 4.13, 95% CI: 1.07–15.99), abortion history (AOR = 7.78, 95% CI: 1.75–18.68), storage time of transfused blood for more than 20 days (AOR = 3.96, 95% CI: 1.03–15.2), and transfused blood increased by 1 unit (AOR = 2.07, 95% CI: 1.21–3.55) were significantly associated with ATR in multivariable logistic regression analysis (Table 4).

Discussion

In the current study, we have evaluated acute transfusion complications and associated factors among 182 adult patients who were transfused blood.

An ATR is an unintended outcome related to blood product transfusion that happens during transfusion and 24 hr after cessation of transfusion.^{5,6} In this study, the incidence of ATR was encountered in 12 patients (6.6%) (95% CI: 3.3–10.7%). This result was in line with previous studies conducted in Bahir Dar, Ethiopia (5.2%),⁸ Bangladesh (3.6%),¹⁹

Table 4 Bivariable and Multivariable Binary Logistic Regression Analysis of Associated Factors of ATRs Among Adult Transfused Patients at DMCSH

Variables		ATR No (%)		COR	AOR (95% CI)	p-value
		Yes	No			
Gender (n=182)	Male	5 (5.1)	94 (94.9)	Ref		
	Female	7 (8.4)	76 (91.6)	1.71 (0.52–5.61)		0.374
Residence (n=182)	Urban	6 (18.2)	27 (81.8)	5.29 (1.58–17.53)	3.87 (1.00–14.88)	0.051
	Rural	6 (4.0)	143 (96.0)	Ref	Ref	
Age years (n=182)	18–35	4 (3.6)	106 (96.4)	Ref	Ref	
	36–45	2 (6.9)	27 (93.1)	1.94 (0.34–11.81)	2.18 (0.33–14.49)	0.420
	≥46	6 (13.9)	37 (86.1)	4.26 (1.14–15.93)	3.62 (0.80–16.37)	0.095
Transfusion history (n=182)	Yes	7 (15.2)	39 (84.8)	4.67 (1.40–15.52)	4.13 (1.07–15.99)	0.040*
	No	5 (3.7)	131 (94.3)	Ref	Ref	
Pregnancy (83)	Yes	5 (7.6)	61 (92.4)	0.62 (0.11–3.44)		0.582
	No	2 (11.8)	15 (88.2)	Ref		
Abortion history (n=66)	Yes	4 (30.8)	9 (69.2)	9.11 (2.31–11.75)	7.78 (1.75–18.68)	0.015*
	No	1 (1.9)	52 (98.1)	Ref	Ref	
Storage time (days) (n=182)	≤20	4 (3.2)	121 (96.8)	Ref	Ref	
	>20	8 (14.0)	49 (86.0)	4.89 (1.41–17.01)	3.96 (1.03–15.2)	0.045*
Transfused units (=182)				2.42 (1.44–4.06)	2.07 (1.21–3.55)	0.008*
Qualifications (n=182)	Specialist	3 (5.5)	52 (94.5)	1.21 (0.12–12.32)		0.871
	General practitioner	8 (7.6)	97 (92.4)	1.73 (0.21–14.60)		0.614
	Nurses/midwifery	1 (4.6)	21 (95.4)	Ref		

Note: *Significantly associated factors.

Abbreviations: Ref, Reference categorical variables; COR, crude odd ratio; AOR, adjusted odd ratio; CI, confidence interval; ATR, Acute Transfusion Reaction; No, number.

Sweden (7.9%),²⁷ Tzu-Chi Hospital, Taiwan (3.5%),²⁴ Belgaum (4.4),²⁸ Kano, and North-Western Nigeria (3.6%).²⁹ The result of this study was higher than studies conducted in Iran (0.95),¹⁸ India (0.96%),¹ Namibia (0.2%),²¹ Bangalore, India (0.5%),¹⁷ and Democratic Republic of Congo 2.6.²² One of the reasons may be that component transfusion was practiced in the areas mentioned later, especially in Iran, where leukocyte filtration was practiced. In contrast, only whole blood is used in the current study, which has triggering factors for TR.³⁰ The other reason may be that most studies were done based on the report of national hemovigilance, which may decrease the incidence rate. India has an incidence rate of 1 in 1412 in the uploaded and 1 in 743 without consideration of uploaded.³¹

In our study, allergic reactions (50%) and FNHTR (33.33%) were the two most common types of ATRs, which is consistent with studies conducted in Bahir Dar, Ethiopia (65% and 30%),⁸ Iran (49.2%, 37.2%),¹⁸ and Bangladesh (40.5%, 36.4%),¹⁹ respectively. This study's proportion of FNHTR was higher than in India (22.8%),¹ and the Democratic Republic of the Congo (19.2%),²² but lower than in Kano, North-Western Nigeria (90%).²⁹

Recipient immunoglobulin E or non-IgE antibodies to proteins or other allergenic soluble substances in the donor plasma cause the allergic reaction. Due to the infused antigens, such as WBC, which increase the recipient's in vivo cytokine production, a febrile non-hemolytic transfusion reaction developed. The infusion of donor cytokines generated in storage may also affect the start of allergic responses and FNHTR in transfusion recipients.³² Histamine, lipids, complement fragments, and cytokines are examples of potential biological response modifiers. These substances arise during storage in RBC or PLT products or are both linked to TR. There is no clinical proof that cytokines cause ATR since their levels are low in products made from preserved erythrocytes.³³

In this study, AHTR was encountered in one patient with a proportion of (8.6%) from the total of ATR. This is comparable to the study conducted in Iran 6.8%¹⁸ and Bangladesh (9.4%).¹⁹ This patient's cross-match result was compatible in the saline technique but incompatible in IAT, suggesting that AHTR is caused by incompatible blood transfusion.³⁴ Furthermore, TRALI was observed only in one patient, accounting for 8.6% of the total ATR. This patient was presented with respiratory distress and bilateral infiltration in x-ray result. This might be due to the infusion of

plasma containing antibodies against the patient's WBCs. Lung edema and neutrophil buildup are brought on by complement activation and cytokine stimulation.³⁵

In the current study, patients who had previous transfusion histories were 4.13 times more likely to develop ATR than its counterpart. The reason may be due to the immune system's sensitization and production of antibodies to certain blood cell antigens (RBC, WBC, and PLT) after the previous transfusion. This sensitized immune system may cause the development of HTR or FNHTR in a subsequent blood transfusion.^{36,37} According to the American Society of Hematology, alloimmunization to sickle cell disease and thalassemia causes hyper-hemolysis in subsequent transfusions.³⁸ It also causes immune modulation and leads to immune suppression, which increases the risk of pneumonia and sepsis.³⁹ The formation of alloantibodies can directly increase the morbidity and mortality of transfusion-dependent patients by increasing the probability of HTR.⁴⁰ Previous transfusion was found to be significantly associated with TR, similar to studies done in Bahir Dar, Ethiopia⁸ and Kano, north west, Nigeria.²⁹

Additionally, as transfused blood increased by 1 unit, the odds of developing ATR increased by 2.07, which was similar to the study conducted in Bahir Dar, Ethiopia.⁸ Repeated or multi-unit blood transfusions can result in the production of alloantibodies against one or more red cell antigens, which complicates the subsequent transfusions.^{36,37} In multiple (many units of transfusion) biochemicals, cytokines, and antibodies complexity happens leading to TR. Endothelial vasculature may induce recipient neutrophil priming and production of reactive oxygen species (ROS). These changes may produce depletion of available nitric oxide, with subsequent endothelial dysfunction predisposing to morbidity and mortality in the transfused patients.⁴¹ In addition, there might be an incompatibility between the previously transfused blood and the new blood, which leads to the patient developing ATR. Multiple units of transfusion are associated with FNHTR and allergic reactions.³²

Moreover, in pre-transfusion Hb, as Hb increased by 1 g/dl, the odds of patient improvement increased by 69%. When Hb is low, the patient is more likely to receive many units, resulting in complex formation and decreased patient compliance. Studies indicate a negative correlation between pre-transfusion Hb and transfusion complications in Thailand.⁴² Patients' low pre-transfusion hematocrit and the dose of PRBCs administered were risk factors, but not factors in survival.^{39,43}

Patients who had a history of abortion were 7.78 times more likely to develop ATR. During an abortion, fetal antigens may enter maternal circulation, leading to sensitization. When transfusion takes place, the immune system produces antibodies, which cause AHTR or FNHTR.⁴⁴ The finding is similar to the study in Bahir Dar, Ethiopia.⁸

In this study, patients who had received blood stored for more than 20 days had a risk of developing ATR by 3.96 times than its counter. This is similar to a study conducted in Kano, North-Western Nigeria.²⁹ The effect of young versus old blood on patient outcomes is still controversial. Red blood cell storage induces progressive biochemical, biomechanical, and immunological changes that affect RBC viability, survival, oxygen delivery capacity, and the recipient's response to transfusion.⁴¹ ICU patients, cardiac surgery patients and trauma patients with prolonged storage of transfused blood may have a higher mortality rate.⁴⁵ In contrast, the age of transfused PRBC units was not identified as a risk factor for TR.^{39,43} Despite this, in the United States, mortality after massive blood transfusion was no worse in patients transfused with PRBC stored for long periods of time.⁴⁶

Limitations of the Study

Because whole blood is the only blood transfused in the study area, this study used only whole-blood transfusion, which may overestimate the prevalence of transfusion reactions. Antibody screening, identification, and organ function tests were not assessed for those who developed AHTR.

Conclusion and Recommendations

We concluded that the incidence of ATR was higher than the 2014 WHO report in African countries.¹² Patients with a history of transfusion, abortion, blood transfusion older than 20 days, or more than 1 transfusion were associated with developing ATR.

We recommend that clinicians should closely monitor and consider transfused patients who have a history of previous transfusions and abortions. Regardless of these factors, they should strictly adhere to transfused patients who have old blood transfused, or more than 1 unit transfused. Laboratory personnel should implement a crossmatch of at least saline

and IAT to handle TRs due to incompatibility. Additionally, for those suspected of having TRs, antibody screening and identification should be performed. We recommend that researchers look into multi-center cohort studies to see if there are any explanatory factors, outcomes, or ATR subtypes.

Abbreviations

AHG, Anti-Human Globulin; AHTR, Acute Hemolytic Transfusion Reactions; ATR, Acute Transfusion Reaction; CBC, Complete Blood Count; DAT, Direct Anti-Globulin Test; DMCSH, Debre Markos Comprehensive Specialized Hospital; FFP, Fresh Frozen Plasma; FNHTR, Febrile Non-Hemolytic Transfusion Reaction; ICU, Intensive Care Unit; TACO, Transfusion Associated Circulatory Overload; TRALI, Transfusion Related Acute Lung Injury; WHO, World Health Organization.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable requests.

Ethical Approval and Informed Consent

This research was carried out in accordance with the Helsinki Declaration. Ethical approval was obtained from the School of Biomedical and Laboratory Sciences, Research Ethical Review Committee, College of Medicine and Health Sciences, University of Gondar (Reference number SBMLS/189/2014). Before recruitment, the aim, potential benefits, and risks of the study were explained to participants or their attendants. The written informed consent or assent was taken from volunteer participants or attendants, respectively. Furthermore, whenever a TRs or abnormal laboratory results occurred, the patient was linked to responsible health professionals to manage the problem.

Acknowledgments

We want to acknowledge DMCSH staff who helped during data collection. We also thank the study participants for their voluntary participation in the study.

Author Contributions

All authors made a significant contribution to the study reported whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation or all the areas: took part in drafting, revising, critically reviewing the article: gave final approval of the version to be published: have agreed on the journal to which the article to which the article has been submitted and agreed to accountable for all aspects of the work.

Funding

No funded organization.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Krishnamurthy AV, Mathialagan J, Raghavan AV, Srinivasan S. Analysis of patterns of adverse transfusion reactions and management: a novel initiative toward hemovigilance in a teaching hospital of South India. *J Lab Physicians*. 2020;12:133–140.
2. The American Red Cross. *A Compendium of Transfusion Practice Guidelines*, Vol. 4. The American Red Cross; 2021:3–8
3. World Health Organization. *Clinical Transfusion Practice: Guidelines for Medical Interns*, Vol. 2. World Health Organization; 2020:26–33
4. Adewoyin AS, Oyewale OA. Complications of allogeneic blood transfusion: current approach to diagnosis and management. *Int Blood Res Rev*. 2015;3(4):135–151. doi:10.9734/IBRR/2015/17874
5. Jolee T, Suddock KP. Transfusion reactions. In: *NCBI Book Shelf: A Service of the National Library of Medicine, National Institutes of Health*. Stat Pearls Publishing; 2021.
6. sharma S, Sharma P, Lisa N. Transfusion of blood and blood products: indications and complications. *Am Acad Fam Physicians*. 2011;83(6):719–724.

7. Tufa F, Kiya T, Maleko G, et al. Assessment of recipients' characteristics, transfusion appropriateness, and utilization pattern of blood and blood products in Jimma Medical Center, Jimma, Ethiopia. *PLoS One*. 2021;16(4):e0250623. doi:10.1371/journal.pone.0250623
8. Gelaw Y, Woldu B, Melku M. Proportion of acute transfusion reaction and associated factors among adult transfused patients at felege hiwot compressive referral hospital, Bahir Dar, Northwest Ethiopia: a cross-sectional study. *J Blood Med*. 2020;11:227–234. doi:10.2147/JBM.S250653
9. Mahteme Bekele M, Yisak EH, Gebreselassie HA, et al. A cross-sectional study to analyze blood and blood component transfusion practice at tertiary care hospital of Ethiopia. *Clin Audit*. 2021;13:29–35. doi:10.2147/CA.S326707
10. Tamene M, Tsegaye A, Birhanu A, et al. Assessment of transfusion utilization and patient outcomes at the largest referral and university hospital in Addis Ababa, Ethiopia. *ISBT Sci Ser*. 2016;11(1):7–13. doi:10.1111/voxs.12234
11. Tewabe H, Mitiku A, Worku H. Assessment of blood transfusion utilization and patient outcomes at Yekatit-12 Hospital, Addis Ababa, Ethiopia. *J Blood Med*. 2022;13:171. doi:10.2147/JBM.S355178
12. World Health Organization. *Status of Blood Safety in the Who African Region*. Brazzaville: World Health Organization; 2014.
13. Qin Caroline X, Yesanharao Lekha V, Merkel Kevin R, et al. Blood utilization and clinical outcomes in ECMO patients. *Anesth Analg*. 2020;131(3):901. doi:10.1213/ANE.00000000000004807
14. Unal D, Senayli Y, Polat R, et al. Peri-operative blood transfusion in elective major surgery: incidence, indications and outcome - an observational multicentre study. *Blood Transfus*. 2020;18(4):261–279. doi:10.2450/2020.0011-20
15. World Health Organization. *Global Status Report on Blood Safety and Availability*. Geneva: World Health Organization; 2017.
16. Yaddanapudi S, Yaddanapudi L. Indications for blood and blood product transfusion. *Indian J Anaesth*. 2014;58(5):38–42. doi:10.4103/0019-5049.144648
17. Shweta P. Surveillance of transfusion related adverse reactions in a tertiary care centre in Bangalore: a 4-year hemovigilance initiative. *Indian J Hematol Blood Transfus*. 2020;36(4):733–739. doi:10.1007/s12288-020-01312-w
18. Mehrdad P, Mohammad Erfan Z, Atefeh Nasir K, et al. Descriptions of acute transfusion reactions in the teaching hospitals of Kermanshah University of Medical Sciences, Iran. *Int J Hematol Oncol Stem Cell Res*. 2013;7(2):11.
19. Miah SS, Doha MA, Islam A, et al. The first data of hemovigilance: appraised from a single center in Bangladesh. *Glob J Transfus Med*. 2021;2(2):18–23.
20. Harvey AR, Basavaraju SV, Chung KW, Kuehnert MJ. Transfusion-related adverse reactions reported to the National Healthcare Safety Network Hemovigilance Module, United States, 2010 to 2012. *Transfusion*. 2015;55(4):709–718. doi:10.1111/trf.12918
21. Meza BP, Lohrke B, Rea W. Estimation of the prevalence and rate of acute transfusion reactions occurring in Windhoek, Namibia. *Blood Transfus*. 2014;12(3):352. doi:10.2450/2013.0143-13
22. Musumari PM, Mbikayi SM, Srihanaviboonchai K, Techasrivichien T, Tangmunkongvorakul A, Tshilolo L. Symptoms of acute transfusion reactions at a general referral hospital in Kinshasa, Democratic Republic of Congo: a cross-sectional study. *BMJ open*. 2021;11(7):e045081. doi:10.1136/bmjopen-2020-045081
23. Alter HJ, Klein HG. The hazards of blood transfusion in historical perspective. *blood*. 2008;112(7):2617–2626. doi:10.1182/blood-2008-07-077370
24. Yao C-Y, Chien J-H, Chuang H-Y, Ho T-F. Associated factors with acute transfusion reaction from hospital online reporting events: a retrospective cohort study. *J Patient Saf*. 2020;16:e303–e309. doi:10.1097/PTS.0000000000000527
25. CSA. *Central Statistical Agency of the Federal Democratic Republic of Ethiopia*. Vol. 2. Addis Ababa: CSA; 2012.
26. Health informatics. Debre markos comprehensive specialized hospital administrative information; 2021.
27. Thurn L, Wikman A, Westgren M, Lindqvist PG. Incidence and risk factors of transfusion reactions in postpartum blood transfusions. *Blood Adv*. 2019;3(15):2298–2306. doi:10.1182/bloodadvances.2019000074
28. Madhushree D, Metgud MC, Patil K. Retrospective analysis of all patients undergoing blood transfusion in obstetrics at a Tertiary Care Hospital, Belgaum: a cross-sectional study. *Indian J Health Sci Biomed Res*. 2018;11(2):116. doi:10.4103/kleuhsj.kleuhsj_233_17
29. Gwaram BA, Borodo MM, Dutse AI, A K-G. Pattern of acute blood transfusion reactions in Kano, North-Western Nigeria. *Niger J Basic Clin Sci*. 2012;9(1):27–32. doi:10.4103/0331-8540.102110
30. Vasudev R, Sawhney V, Dogra M, Raina TR. Transfusion-related adverse reactions: from institutional hemovigilance effort to National Hemovigilance program. *Asian J Transfus Sci*. 2016;10(1):31–36. doi:10.4103/0973-6247.175391
31. Dhiman Y, Raturi M, Kala M, Kusum A. Significance of mandating a dedicated hemovigilance nurse and objectification of 'imputability' levels. Experience from a hospital-based blood centre in India. *Transfusion clinique et biologique*. 2023;30(1):96–102. doi:10.1016/j.traccli.2022.09.066
32. Pomper GJ. Febrile, allergic, and nonimmune transfusion reactions. *Rossi's Princ Transfus Med*. 2009;2009:826–846.
33. Heddle NM. Pathophysiology of febrile nonhemolytic transfusion reactions. *Curr Opin Hematol*. 1999;6(6):420–426. doi:10.1097/00062752-199911000-00012
34. Webb C, Norris A, Hands K. An acute transfusion reaction. *Clin Med*. 2018;18(1):95–97. doi:10.7861/clinmedicine.18-1-95
35. Fung YL, Silliman CC. The role of neutrophils in the pathogenesis of transfusion-related acute lung injury. *Transfus Med Rev*. 2009;23(4):266–283. doi:10.1016/j.tmr.2009.06.001
36. Bhuvu DK, Vachhani JH. Red cell alloimmunization in repeatedly transfused patients. *Asian J Transfus Sci*. 2017;11(2):115–120. doi:10.4103/0973-6247.214347
37. Bowman Z, Fei N. Single versus double-unit transfusion: safety and efficacy for patients with hematologic malignancies. *Eur J Haematol*. 2019;102(5):383–388. doi:10.1111/ejh.13211
38. Chou S, Alsawas M, Fasano R, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. *Blood Adv*. 2020;4(2):327–355. doi:10.1182/bloodadvances.2019001143
39. Blajchman MA. Immunomodulation and blood transfusion. *Am J Ther*. 2002;9(5):389–395. doi:10.1097/00045391-200209000-00005
40. Chonat S, Quarmyne M-O, Bennett CM, et al. Contribution of alternative complement pathway to delayed hemolytic transfusion reaction in sickle cell disease. *Haematologica*. 2018;103(10):e483. doi:10.3324/haematol.2018.194670
41. Obrador R, Musulin S, Hansen B. Red blood cell storage lesion. *J Vet Emerg Crit Care*. 2015;25(2):187–199. doi:10.1111/vec.12252
42. Wanchaitanawong W, Tantiworawit A, Piriyahtorn P, et al. The association between pre-transfusion hemoglobin levels and thalassemia complications. *Hematology*. 2021;26(1):1–8. doi:10.1080/16078454.2020.1856513
43. Holowaychuk MK, Leader JL, Monteith G. Risk factors for transfusion-associated complications and nonsurvival in dogs receiving packed red blood cell transfusions: 211 cases (2008–2011). *J Am Vet Med Assoc*. 2014;244(4):431–437. doi:10.2460/javma.244.4.431

44. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. *Blood*. 2019;133(17):1821–1830. doi:10.1182/blood-2018-08-833962
45. Ng MS, Ng AS, Chan J, Tung JP, Fraser JF. Effects of packed red blood cell storage duration on post-transfusion clinical outcomes: a meta-analysis and systematic review. *Intensive Care Med*. 2015;41(12):2087–2097. doi:10.1007/s00134-015-4078-5
46. Bautista A, Wright TB, Meany J, et al. Red cell storage duration does not affect outcome after massive blood transfusion in trauma and nontrauma patients: a retrospective analysis of 305 patients. *Hindawi*. 2017;2017:3718615.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Pharmacokinetic-Pharmacodynamic Comparison of Recombinant and Plasma-Derived von Willebrand Factor in Patients with von Willebrand Disease Type 3

Alexander Bauer, Sofia Friberg-Hietala, Giovanni Smania & Martin Wolfsegger

To cite this article: Alexander Bauer, Sofia Friberg-Hietala, Giovanni Smania & Martin Wolfsegger (2023) Pharmacokinetic-Pharmacodynamic Comparison of Recombinant and Plasma-Derived von Willebrand Factor in Patients with von Willebrand Disease Type 3, Journal of Blood Medicine, , 399-411, DOI: [10.2147/JBM.S395845](https://doi.org/10.2147/JBM.S395845)

To link to this article: <https://doi.org/10.2147/JBM.S395845>



© 2023 Bauer et al.



Published online: 13 Jun 2023.



Submit your article to this journal



Article views: 333



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Pharmacokinetic-Pharmacodynamic Comparison of Recombinant and Plasma-Derived von Willebrand Factor in Patients with von Willebrand Disease Type 3

Alexander Bauer¹, Sofia Friberg-Hietala², Giovanni Smania², Martin Wolfsegger¹

¹Statistical and Quantitative Sciences, Baxalta Innovations GmbH, a Takeda Company, Vienna, Austria; ²Pharmetheus AB, Uppsala, Sweden

Correspondence: Alexander Bauer, Baxalta Innovations GmbH, a Takeda company, Donau-City-Straße 7, Vienna, A-1220, Austria, Tel +43 1 201002472997, Email alexander.bauer@takeda.com

Background: Recombinant von Willebrand factor (rVWF, vonicog alfa, Vonvendi/Veyvondi, Takeda Pharmaceuticals USA, Lexington, MA) and several plasma-derived VWF/factor VIII (pdVWF/FVIII) concentrates are available for treating bleeding episodes in patients with von Willebrand disease (VWD).

Purpose: To develop population pharmacokinetic (PK)/pharmacodynamic (PD) models that describe VWF:ristocetin cofactor (VWF:RCo) activity and its relationship with FVIII activity (FVIII:C) over time following intravenous administration of either rVWF or a pdVWF/FVIII concentrate (VWF:RCo/FVIII:C 2.4:1) in patients with VWD; to use the final PK/PD models for an in silico comparison of rVWF and pdVWF/FVIII.

Methods: The population PK model for rVWF was based on data from four clinical studies in which rVWF was administered to adult patients with VWD type 1, 2 or 3 (phase 1: NCT00816660; phase 3: NCT01410227 and NCT02283268) or severe hemophilia A (phase 1: EudraCT 2011-004314-42). The PK and PK/PD models for pdVWF/FVIII were based on data from the phase 1 study (NCT00816660) in patients with type 3 VWD who received either rVWF plus recombinant FVIII (rFVIII, octocog alfa, ADVATE®, Takeda Pharmaceuticals USA, Lexington, MA, USA) or pdVWF/FVIII.

Results: There was a marked difference in clearance following rVWF administration compared with pdVWF/FVIII in type 3 VWD, leading to a ~1.75 longer mean residence time (ie, persistence of VWF:RCo activity in the body) and half-life for rVWF versus pdVWF/FVIII. Simulations showed that following repeated administration of rVWF (50 IU/kg), a FVIII:C activity of >40 IU/dL can be maintained for the full 72 h dosing interval.

Conclusion: The slower elimination of VWF:RCo following rVWF administration results in a prolonged effect on FVIII turnover compared with pdVWF/FVIII administration.

Keywords: factor VIII, pharmacodynamics, pharmacokinetics, plasma-derived, recombinant, von Willebrand factor

Introduction

Von Willebrand disease (VWD) is an inherited bleeding disorder characterized by qualitative or quantitative defects in von Willebrand factor (VWF), a large multimeric glycoprotein that mediates platelet adhesion and stabilizes factor VIII (FVIII) in the circulation.¹⁻⁴ Recombinant VWF (rVWF, vonicog alfa, Vonvendi® [USA, Japan, Australia] / VEYVONDI™ [EU, UK, Switzerland], Takeda Pharmaceuticals USA, Lexington, MA, USA) has demonstrated efficacy with a favorable safety profile when used for on-demand treatment of hemorrhage and the prevention and treatment of surgical bleeding in adults with VWD.⁵⁻⁸ The efficacy and safety of prophylaxis with rVWF in patients with severe VWD has also been assessed in a phase 3, open-label, nonrandomized trial.⁹ In that study, rVWF reduced the rate of treated, spontaneous bleeding events in patients previously receiving on-demand VWF therapy by 91.5%.⁹ Several human plasma-derived VWF (pdVWF)/FVIII concentrates have been approved for the treatment of bleeding episodes in patients with VWD, and their efficacy, safety, and pharmacokinetic (PK) data are published.¹⁰⁻¹² Of these, Haemate P®/Humate-P® (pdFVIII/VWF complex [Human]; CSL Behring GmbH, Marburg,

Germany: VWF: ristocetin cofactor [RCo]/ factor VIII activity [FVIII:C] 2.4:1) is a widely used pdVWF/FVIII concentrate for the treatment of VWD that is effective in the prevention and control of bleeding in patients with VWD.^{12–14}

Intravenous administration of rVWF corrects VWF platelet binding activity (as measured by VWF:RCo), and increases FVIII:C to hemostatically effective levels within 6 hours (h) in most patients.^{5,6,8} rVWF replaces VWF without necessarily requiring the coadministration of exogenous FVIII in most of the cases.¹⁵ All currently licensed pdVWF products, except for Wilfactin®/Willfact® (LFB, France), contain FVIII, with a ratio of VWF:RCo/FVIII:C varying from 1.1 to 2.54.¹⁶ As a result, the pdVWF from these products will stabilize both exogenously administered FVIII (if the product contains it), as well as the endogenous FVIII pool. This may result in FVIII accumulation, especially in patients undergoing surgery who have increased levels of FVIII:C postoperatively as part of the physiological response to the procedure.^{17,18}

The objective of this work was to develop population PK/pharmacodynamic (PD) models that describe VWF:RCo activity and its relationship with FVIII:C over time following intravenous administration of either rVWF or pdVWF/FVIII (VWF:RCo/FVIII:C 2.4:1) in patients with VWD. Then, use the final PK/PD models for an in silico comparison of rVWF and pdVWF/FVIII. An improved understanding of the PK and PK/PD of these products should help with the individualized treatment of patients with VWD based on PK/PD-guided dosing strategies.

Methods

Patients and Data Collection

The population PK model for rVWF was based on four clinical studies in which rVWF was administered in adult patients with VWD type 1, 2 or 3 (phase 1: NCT00816660;¹⁹ phase 3: NCT01410227⁶ and NCT02283268)⁷ or severe hemophilia A (phase 1: EudraCT 2011–004314-42) (Table 1 and Table 2). Patients who developed inhibitory antibodies to VWF or FVIII (titer ≥0.6 Bethesda units using the Bethesda assay) or were co-administered plasma-derived replacement therapy (treatment with pdVWF/FVIII in NCT00816660)¹⁹ were excluded from the analysis. In addition, observations associated with a bleeding event were excluded. VWF:RCo and FVIII:C were measured before dosing and over various time points up to 120 h after intravenous rVWF infusion. Because patients in the phase 1 studies received concomitant exogenous FVIII concentrates, the population PK/PD model for rVWF was developed using data from the two phase 3 studies. FVIII activity was quantified by the one-stage clotting assay in all studies.

Table 1 Clinical Studies Contributing Data to the Development of the Population PK/PD Models for rVWF and pdVWF/FVIII

Study Number	Study Design	Study Population	rVWF Dosage Regimen	Patients with VWF:RCo Observations, n (m) ^{a,b}	Patients with FVIII Observations, n (m) ^a
Study 070701, NCT00816660 ¹⁹	Phase 1, randomized, single-blind, dose escalation	Severe types 1 and 3 VWD	rVWF:rFVIII at 2–50 IU/kg VWF:RCo	29 (479)	-
Study 071001, NCT01410227 ⁶	Phase 3, multicenter, open-label, on-demand treatment	Severe types 1, 2A, 2N and 3 VWD	rVWF 50 IU/kg or 80 IU/kg VWF:RCo	31 (694)	30 (475)
Study 071101, NCT02283268 ⁷	Phase 3, open-label, uncontrolled, nonrandomized, surgery	Severe types 1, 2A, 2B, 2M, 3 VWD	Dose/frequency of rVWF dependent on type of surgery	11 (241)	11 (211)
Study 071104, EudraCT 2011–004314-42 ^c	Phase 1, uncontrolled, nonrandomized, multicenter, proof of concept	Severe hemophilia A (FVIII activity <1%)	50 IU/kg rFVIII alone, then in combination with 10 IU/kg rVWF, then in combination with 50 IU/kg rVWF	10 (250)	-

Notes: ^an, number of patients; m, number of samples for the rVWF population PK/PD model. ^bTwo patients were included in both study NCT00816660 and NCT01410227. These patients were counted once when summing up the total. ^cEudraCT 2011–004314-42 was registered on EudraCT but is not publicly available as it is a phase I study. **Abbreviations:** FVIII, factor VIII; PD, pharmacodynamic; PK, pharmacokinetic; rFVIII, recombinant FVIII; rVWF, recombinant von Willebrand factor; VWD, von Willebrand disease; VWF:RCo, von Willebrand factor:ristocetin cofactor.

Table 2 rVWF Population PK and PK/PD Models: Patient Baseline Characteristics

	VWF:RCo PK Model Population	FVIII PK/PD Model Population
No. of patients	79	41
No. of plasma samples	1664	686
rVWF dose range, IU/kg	2–80	5–80
LLOQ, IU/dL	1 and 8 ^a	1
Continuous covariates, median (range)		
Age, years	35 (18–70)	36 (18–70)
Body weight, kg	74 (43.8–145)	72.5 (45–143)
Hematocrit, L/L	0.417 (0.310–0.480)	0.402 (0.31–0.48)
Categorical variables, n (%)		
Sex		
Male	46 (58)	20 (49)
Female	33 (42)	21 (51)
Race		
White	73 (92)	38 (93)
Asian	6 (8)	3 (7)
Disease type		
Type 1 VWD	5 (6)	3 (7)
Type 2 VWD ^b	7 (9)	7 (17)
Type 3 VWD	57 (72)	31 (76)
Severe hemophilia A ^c	10 (13)	0 (0)

Notes: ^aThe LLOQ for VWF:RCo was 1 IU/dL in study NCT00816660 and 8 IU/dL in studies NCT01410227, NCT02283268, and EudraCT2011-004314-42. ^bVWD type 2A (n=5), type 2B (n=1) and type 2M (n=1). ^cThe cohort of patients with hemophilia A was retained for the development of the rVWF population PK model because, after accounting for individual endogenous VWF:RCo levels, no PK differences between the different VWD types and hemophilia A were observed (see PK Model for rVWF results section).

Abbreviations: FVIII, factor VIII; LLOQ, lower limit of quantification; PD, pharmacodynamic; PK, pharmacokinetic; rVWF, recombinant von Willebrand factor; VWD, von Willebrand disease; VWF:RCo, von Willebrand factor:ristocetin cofactor.

The population PK and PK/PD models of pdVWF/FVIII were based on data in patients with type 3 VWD from a single cohort (n=22) of a phase 1, dose escalation study (NCT00816660)¹⁹ (Table 1 and Table 2). Patients received single doses of either rVWF and recombinant FVIII (rFVIII; octocog alfa, ADVATE[®], 50 IU/kg VWF:RCo/38.5 IU/kg FVIII:C) or pdVWF/FVIII (Humate-P, 50 IU/kg VWF:RCo, VWF:RCo/FVIII:C 2.4:1) in a random, crossover cohort design. VWF:RCo and FVIII:C were measured over various time points up to 96 h following intravenous pdVWF/FVIII infusion.¹⁹ Measurements following administration of rVWF and rFVIII were not included in the analysis. Measurements at screening and at the end of the study (30 days × 24 h from the last infusion) were also excluded due to uncertain dosing history at these times. The lower limit of quantification (LLOQ) for VWF:RCo and FVIII:C was 1 IU/dL for all included observations in study NCT00816660.

Model Development

PK and PK/PD models were developed for rVWF (full details in the [Supplementary Methods](#) and [Supplementary Table 1](#)) and then used to develop models for pdVWF/FVIII. Model development and evaluation were performed in accordance with United States Food and Drug Administration and European Medicines Agency guidelines^{20,21} and were based on

a prespecified statistical analysis plan. Population models were developed using nonlinear mixed-effects modeling as implemented in NONMEM software (NONMEM® version 7.3.0 or 7.4.0; ICON Development Solutions, Ellicott City, MD, USA).²² Data assembly and graphical analyses were performed using R version 3.5.3.²³

The final population PK and PK/PD models for the respective products were used to simulate VWF:RCo or FVIII activity–time profiles following repeated administration of rVWF and pdVWF/FVIII (VWF:RCo/FVIII:C 2.4:1). For this analysis, a typical patient with type 3 VWD was defined as having a body weight of 75 kg and a hematocrit level of 0.4 L/L based on the central tendency of the study population.

Results

Population Characteristics

A total of 1664 VWF:RCo samples from 79 patients treated with rVWF in the four clinical studies were available for the PK analysis. Patients treated with rVWF in the two phase 3 VWD studies (n=41) provided 686 FVIII samples for the PK/PD analysis (Table 1). Data from seven patients with antibodies to VWF or FVIII were excluded from the analyses (n=4 binding antibodies to VWF; n=1 inhibitory antibodies to FVIII; n=2 binding antibodies to FVIII IgG). Four VWF:RCo and FVIII:C observations associated with a bleeding event and 308 VWF:RCo and FVIII:C observations collected after the coadministration of plasma-derived therapy were also excluded. The VWF:RCo and FVIII:C analysis populations were heterogeneous with respect to demographics and clinical characteristics (Table 2).

A total of 281 samples were available for VWF:RCo and FVIII:C following administration of pdVWF/FVIII to 20 patients. Two patients were excluded from the analysis: one patient had no PK/PD measurements following administration of pdVWF/FVIII and one patient dropped out prior to receiving the pdVWF/FVIII dose. Details of patient baseline characteristics of the pdVWF/FVIII analysis population are shown in Table 3.

Table 3 pdVWF/FVIII Population PK and PD Models: Patient Baseline Characteristics

	VWF:RCo PK Model Population	FVIII PK/PD Model Population
No. of patients	20	20
No. of samples	281	281
No. of samples >LLOQ	232	281
No. of samples <LLOQ	49	0
Continuous covariates, median (range)	VWF:RCo PK and FVIII PD	
Age, years	30 (18–60)	
Body weight, kg	66.3 (43.8–132)	
Hematocrit, L/L	0.406 (0.334–0.483)	
Categorical variables, n (%)		
Sex		
Male	9 (45)	
Female	11 (55)	
Race		
White	20 (100)	
Disease type		
Type 3 VWD	20 (100)	

Abbreviations: FVIII, factor VIII; LLOQ, lower limit of quantification; PD, pharmacodynamic; pdVWF/FVIII, plasma-derived von Willebrand factor/FVIII; PK, pharmacokinetic; VWD, von Willebrand disease; VWF:RCo, von Willebrand factor:ristocetin cofactor.

PK Model for rVWF

One- and two-compartment disposition models were evaluated for VWF:RCo PK, accounting for endogenous background in VWF:RCo. The final model was a two-compartment model, parametrized in terms of clearance, intercompartmental clearance, central volume of distribution, and volume of distribution for the peripheral compartment. Parameters were allometrically scaled to body weight. As expected, the volume of the central compartment (anticipated to represent blood volume) decreased with increasing hematocrit. Once the individual endogenous VWF:RCo levels were accounted for, no PK differences were observed between the different VWD types and hemophilia A ([Supplementary Results](#)).

PK/PD Model for rVWF

The population PK/PD model was an indirect response model in which VWF:RCo inhibited FVIII elimination, accounting for the delayed FVIII response in relation to rVWF levels ([Supplementary Figures 1 and 2](#)). Model evaluation demonstrated that there was good agreement between individual model predictions and observations of VWF:RCo and FVIII:C data after rVWF doses ([Figure 1](#)).

The simulated VWF:RCo and FVIII activities following a single rVWF dose of 50 IU/kg and following repeated administration every 72 h, stratified by VWD type, are shown in [Figure 2A and B](#). Summary measures of FVIII exposure were computed from the simulated profiles in terms of area under the activity–time curve during the dosing interval (AUC_{72}) and maximum activity (C_{max}) after a single dose and at steady state, and their relationship with VWF:RCo clearance is shown in [Figure 2C and D](#). The simulations at steady state indicated that the median FVIII:C in patients with type 3 VWD was between 40 and 100 IU/dL, and that at least 80% and 97.5% of the treated population would achieve maximal FVIII plasma levels below 150 and 250 IU/dL, respectively.

PK Model for pdVWF/FVIII

The starting point for the population PK model of pdVWF/FVIII was the PK model developed for rVWF; detailed results of the PK modeling for pdVWF/FVIII are provided in the [Supplementary Results](#). Goodness-of-fit plots demonstrated that the model for pdVWF/FVIII adequately predicted the observed VWF:RCo ([Figure 3A](#)), and FVIII:C ([Figure 3B](#)), respectively. Subsequently, there is no indication of a marked model misspecification in these plots.

The primary PK parameter estimates for the pdVWF/FVIII model are presented alongside estimates for the rVWF model in [Table 4](#). There was a marked difference in clearance between the rVWF model and pdVWF/FVIII model (2.10 dL/h vs 4.14 dL/h, respectively), which led to a 1.76 longer mean residence time (MRT, which represents the persistence

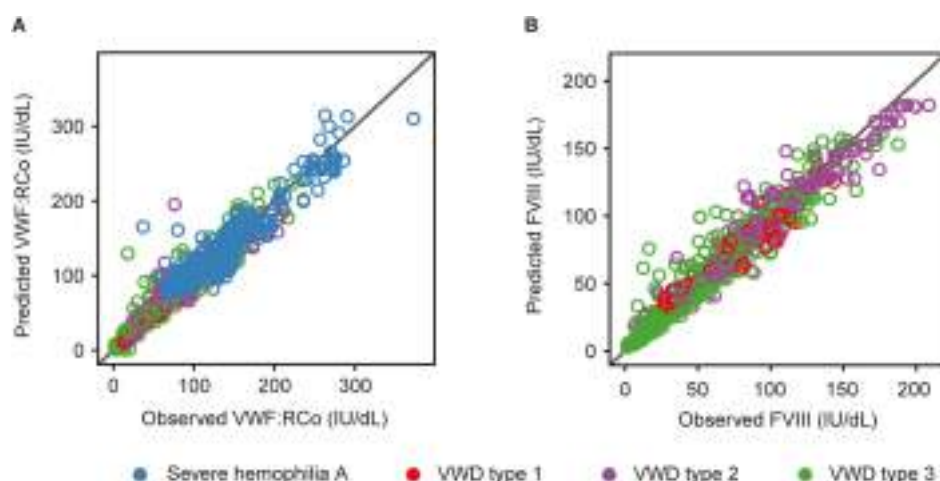


Figure 1 Goodness-of-fit plots for the final population PK and PK/PD models of rVWF. Observed versus individual predictions are shown for **(A)** VWF:RCo and **(B)** FVIII:C data, colored by disease type. The diagonal line depicts the line of identity.

Abbreviations: FVIII, factor VIII; FVIII:C, FVIII activity; PD, pharmacodynamic; PK, pharmacokinetic; rVWF, recombinant von Willebrand factor; VWF:RCo, von Willebrand factor:ristocetin cofactor; VWD, von Willebrand disease.

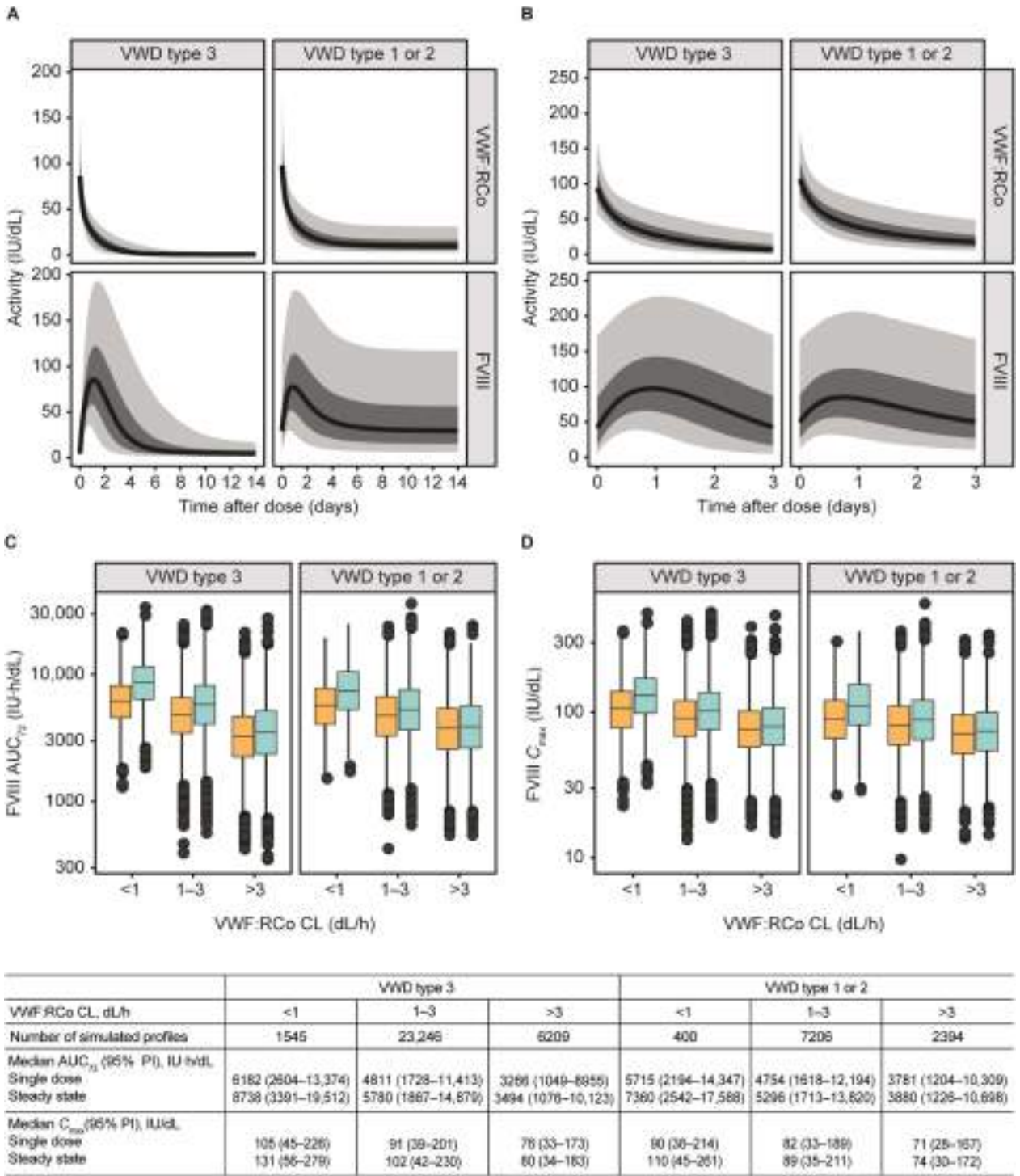


Figure 2 PK/PD model simulations of VWF:RCo and FVIII:C activity following rVWF dosing by VWD type. Simulated VWF:RCo and FVIII activity–time profiles following (A) a single rVWF dose of 50 IU/kg and (B) at steady state after dosing every 72 h. The solid line depicts the median simulated profile, and the dark and light shaded areas represent the 60% and 95% PIs, respectively. The distribution of (C) simulated FVIII AUC₇₂ and (D) C_{max} after a single dose (orange) and at steady state (turquoise) for different ranges of VWF:RCo clearance (the y-axis is displayed in a logarithmic scale); summary statistics for AUC₇₂ and C_{max} are reported in the table.

Abbreviations: AUC₇₂, area under the activity–time curve during the dosing interval; CL, clearance; C_{max}, maximum activity; FVIII, factor VIII; FVIII:C, FVIII activity; PI, prediction interval; PD, pharmacodynamic; PK, pharmacokinetic; rVWF, recombinant von Willebrand factor; VWF:RCo, von Willebrand factor:ristocetin cofactor; VWD, von Willebrand disease.

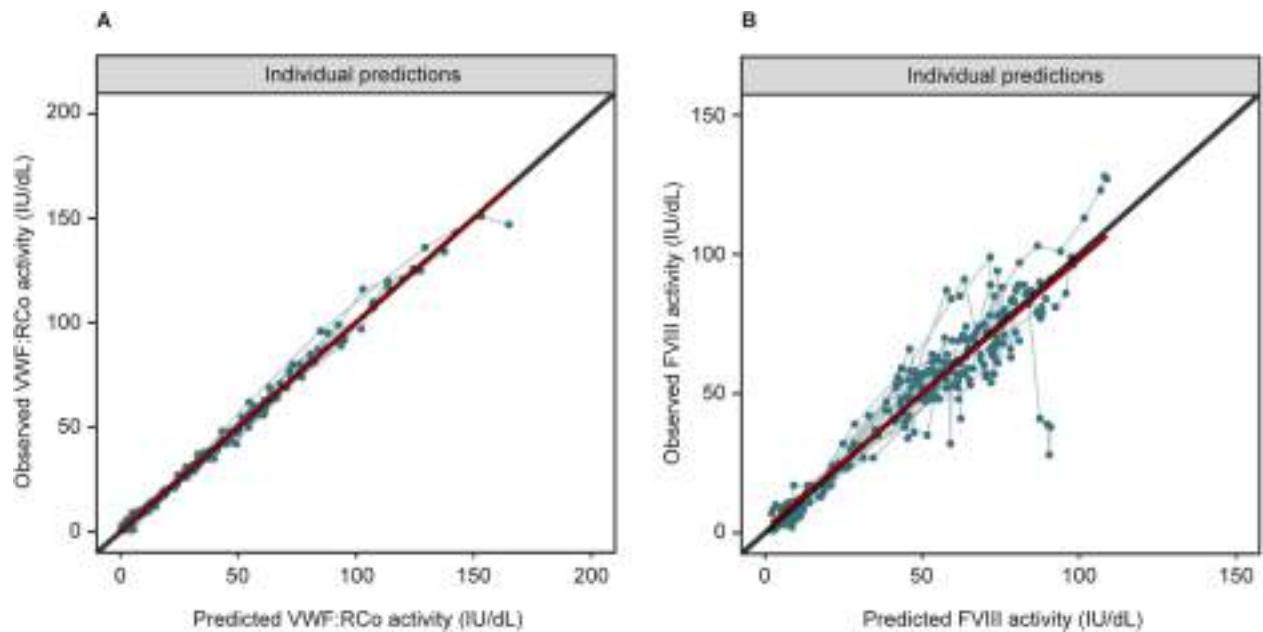


Figure 3 Goodness-of-fit plots for the final population PK and PK/PD models for pdVWF/FVIII. Observed versus individual predictions for **(A)** VWF:RCO and **(B)** FVIII:C data. Individual data points are indicated by dots and the points for each individual are connected with a line. The black diagonal line depicts the line of identity. The red smooth (gam) lines are close to this line indicating that no bias is present. Values below the limit of quantification are not included in the plots.

Abbreviations: FVIII, factor VIII; FVIII:C, FVIII activity; PD, pharmacodynamic; pdVWF/FVIII, plasma-derived von Willebrand factor/FVIII; PK, pharmacokinetic; VWF:RCO, von Willebrand factor:ristocetin cofactor.

of VWF:RCO activity in the body) and 1.74 times longer half-life ($t_{1/2}$) for rVWF than for pdVWF/FVIII. The VWF:RCO AUC_{0-inf} was estimated to be 1.97 times greater following rVWF administration than after pdVWF/FVIII.

The estimated interindividual variability (IIV) in VWF:RCO clearance following pdVWF/FVIII administration was 23.5% versus 40.1% following rVWF administration. There was considerable uncertainty in the estimated IIV for the pdVWF/FVIII model, with a relative standard error of 38.3% versus 9.9% for the rVWF model.

PK/PD Model for pdVWF/FVIII

The starting point for the population PK/PD model of pdVWF/FVIII was the indirect response model developed for rVWF; additional details are provided in the [Supplementary Results](#). Parameter estimates of the final PK/PD model are shown in [Table 4](#). Estimated maximum inhibition (I_{max}) values were similar in both the rVWF and pdVWF/FVIII models, but activity level at half maximum inhibition (IC_{50}) was lower in the pdVWF/FVIII model than in the rVWF model (0.0577 IU/dL vs 0.0658 IU/dL). A volume of distribution term for FVIII (FVIII V) was added to account for the distribution of the pdFVIII dose. The FVIII V was estimated to be 32.9 dL, which is in line with a previous report (32.8 dL).²⁴

PK and PK/PD Model Simulations

PK/PD model simulations of the typical VWF:RCO and FVIII:C profiles following repeated administration of rVWF 50 IU/kg or pdVWF/FVIII 50 IU/kg every 72 h are shown in [Figure 4A](#). There was a modest accumulation of VWF:RCO and FVIII:C with both treatments. The simulations suggest that a FVIII:C above 40 IU/dL can be maintained for the full 72 h dosing interval for a typical patient at this dose of rVWF, in line with recommendations from the European Medicines Agency.²⁵

Additional simulations with an irregular dosing interval of 72 h and 96 h are shown in [Figure 4B](#), reflecting how prophylactic rVWF treatment of patients with VWD could be administered in clinical practice. FVIII activity–time profiles for rVWF followed a similar trend as that for regular dosing (interval of 72 h), and as expected, only trough levels in the 96 h interval were below 40 IU/dL (unlike in hemophilia A,²⁶ FVIII trough levels are not well established in

Table 4 Parameter Estimates of the Population PK (VWF:RCo) and PK/PD (FVIII) Models

Parameter	rVWF Model		pdVWF/FVIII Model	
	Point Estimate	RSE (%) ^a	Point Estimate	RSE (%)
VWF:RCo PK model				
CL, dL/h	2.10	8.10	4.14	12.7
V _c , dL	43.5	4.98	47.0	16.7
Q, dL/h	2.29	12.2	4.47	12.3
V _p , dL	15.8	4.47	19.3	3.12
E _{VWF} VWD type 3, IU/dL	0.500	(FIX)	0.500	(FIX)
WT effect on CL and Q	0.750	(FIX)	0.750	(FIX)
WT effect on V _c and V _p	1.00	(FIX)	1.00	(FIX)
Hematocrit effect on V _c	−0.334	37.5	−0.334	(FIX)
IIV CL, CV ^b	0.401	9.88	0.235	38.3
IIV V _c , CV ^b	0.292	10.7	0.373	51.7
Proportional RUV, CV ^b	0.138	1.26	0.0479	14.1
Additive RUV, IU/dL ^b	2.80	2.08	1.48	12.4
FVIII PK/PD model				
Baseline FVIII, IU/dL ^{c,d}	0.500	–	0.500	(FIX)
k _{out} , h ^{−1} c	15.9	–	15.9	(FIX)
I _{max}	0.998	–	0.994	0.0378
IC ₅₀ , IU/dL	0.0658	–	0.0577	20.4
Hematocrit effect on baseline FVIII	−0.571	–	−0.571	(FIX)
FVIII V, dL	–	–	32.9	1.65
IIV baseline FVIII, CV	0.296	–	0.215	44.4
IIV IC ₅₀ , CV	0.588	–	0.542	29.4
Proportional RUV, CV	0.190	–	0.172	2.50
Additive RUV, IU/dL	1.55	–	2.91	15.5

Notes: ^aThe precision in parameter estimates for the rVWF PK/PD model was estimated using a bootstrap method, which showed that the population estimates of the final model were within the 2.5th to 97.5th percentile obtained from the bootstrap analysis (data not shown). ^bRSE reported on the approximate standard deviation scale. ^cParameter was fixed in the pdVWF/FVIII model. ^dThe estimated baseline FVIII:C represents the theoretical baseline FVIII:C assuming that no VWF is affecting k_{out}.

Abbreviations: CL, clearance; CV, coefficient of variation; E_{VWF}, endogenous von Willebrand factor; FVIII, factor VIII; FVIII:C, FVIII activity; IC₅₀, activity level at half maximum inhibition; IIV, interindividual variability; I_{max}, maximum inhibition; k_{out}, first-order removal; PD, pharmacodynamic; pdVWF/FVIII, plasma-derived von Willebrand factor/FVIII; PK, pharmacokinetic; Q, intercompartmental clearance; RSE, relative standard error; RUV, residual unexplained variability; rVWF, recombinant von Willebrand factor; V_c, central volume of distribution; V_p, peripheral volume of distribution; FVIII V, volume of distribution for FVIII; VWD, von Willebrand disease; VWF:RCo, von Willebrand factor:ristocetin cofactor; WT, weight.

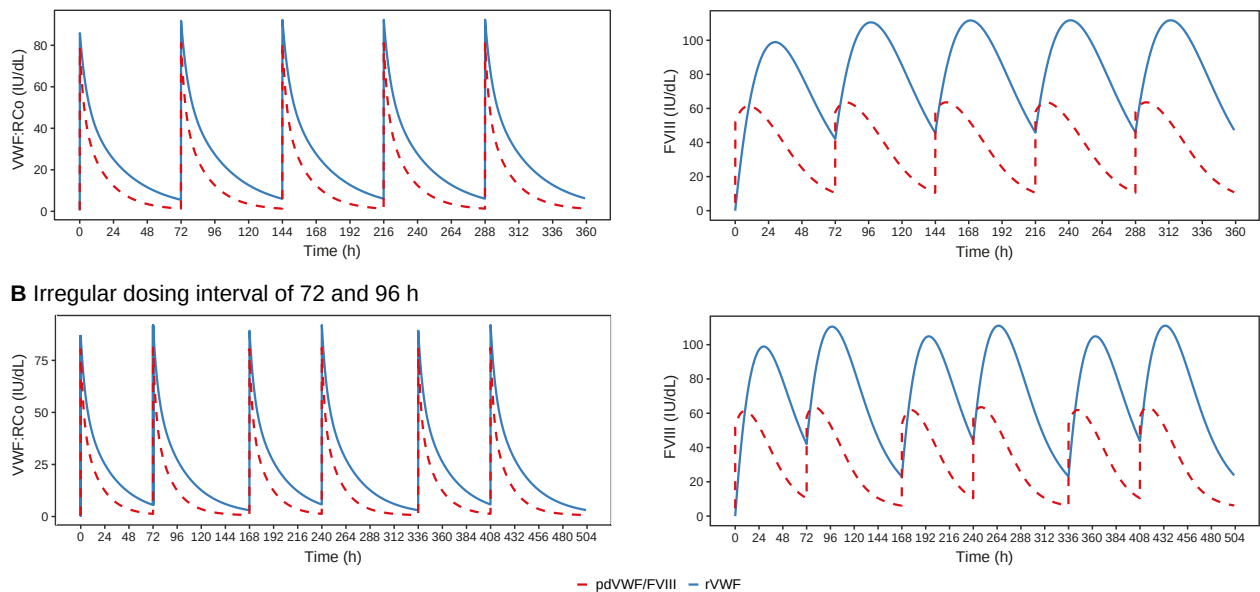
A Regular dosing interval of 72 h

Figure 4 PK/PD model simulations of VWF:RCo and FVIII activity following repeated administration of rVWF 50 IU/kg (blue line) or pdVWF/FVIII 50 IU/kg (VWF:RCo/FVIII: C 2.4:1; red dashed line) at (A) regular and (B) irregular dosing intervals in a 75 kg individual with a hematocrit of 0.4 L/L.

Abbreviations: FVIII, factor VIII; PD, pharmacodynamic; pdVWF/FVIII, plasma-derived von Willebrand factor/FVIII; PK, pharmacokinetic; rVWF, recombinant von Willebrand factor; VWF:RCo, von Willebrand factor:ristocetin cofactor.

VWD). The ratio of the elimination FVIII $t_{1/2}$ for rVWF treatment to the FVIII $t_{1/2}$ for pdVWF/FVIII ranged from 2.4 to 3.8 during a dosing interval of 72 h, demonstrating a marked difference in the impact on FVIII:C between the two products (Figure 5A and B).

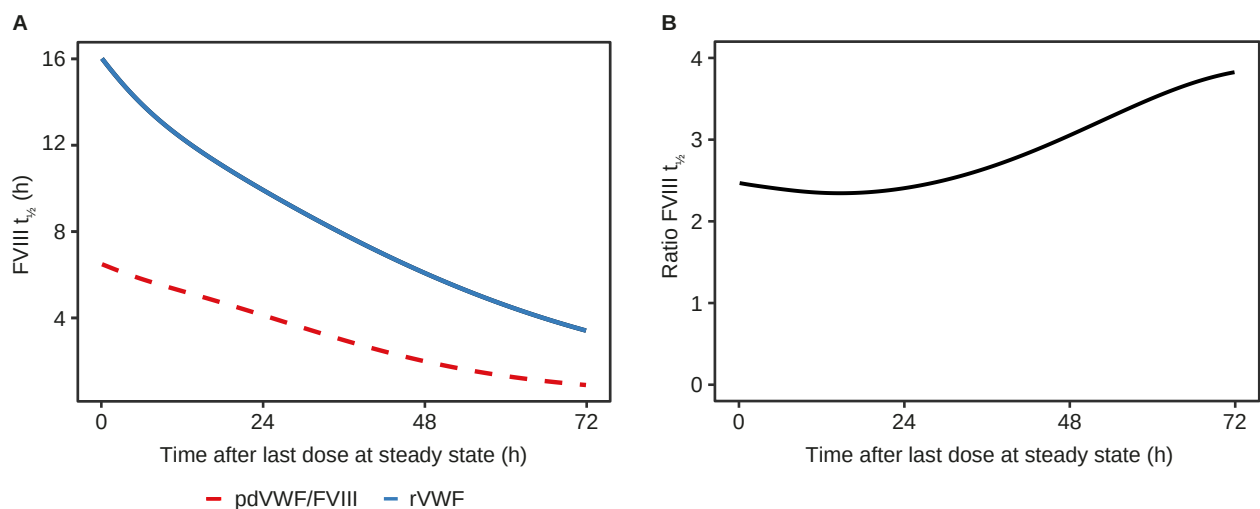


Figure 5 Model predicted (A) FVIII $t_{1/2}$ and (B) ratio of FVIII $t_{1/2}$ for rVWF to FVIII $t_{1/2}$ for pdVWF/FVIII during a dosing interval at steady state following repeated administration of rVWF 50 IU/kg (blue line in A) or pdVWF/FVIII 50 IU/kg (VWF:RCo/FVIII: C 2.4:1; red dashed line in A) every 72 h to a 75 kg individual with a hematocrit of 0.4 L/L.

Abbreviations: FVIII, factor VIII; FVIII:C, FVIII activity; pdVWF/FVIII, plasma-derived von Willebrand factor/FVIII; rVWF, recombinant von Willebrand factor; $t_{1/2}$, half-life; VWF, von Willebrand factor; VWF:RCo, von Willebrand factor:ristocetin cofactor.

Discussion

A personalized approach to the management of VWD has several benefits, as previously discussed.^{27,28} A population PK/PD model in which individual PK data can be more easily considered when making dosage recommendations in VWD is important to facilitate this approach. The population PK and PK/PD models for rVWF developed in this study predict VWF:RCo and FVIII activity over time in patients with severe VWD type 1, 2 and 3 treated with rVWF. Patients with VWD type 3 have a total or near-total absence of VWF in the circulation, while patients with severe VWD types 1 and 2 have reduced VWF in the circulation.^{1,29} The population PK and PK/PD models developed for rVWF were refined to fit pdVWF/FVIII (VWF:RCo/FVIII:C 2.4:1) data for a similar VWD type 3 patient population. The population PK for VWF:RCo following a single dose of rVWF or pdVWF/FVIII was adequately described by a two-compartment disposition model with first-order elimination from the central compartment ([Supplementary Figure 1](#)). This type of modeling is often used in PK analyses to represent the distribution of a drug in the body occurring between two compartments: a central compartment (commonly representing blood and well-perfused organs), and a peripheral compartment (commonly representing poorly perfused tissues).³⁰ Our findings demonstrated a slower clearance of VWF:RCo following rVWF compared with pdVWF/FVIII as indicated by longer MRT and $t_{1/2}$ values and a larger VWF:RCo AUC_{0-inf} with rVWF; this results in a prolonged effect on FVIII turnover with rVWF compared with pdVWF/FVIII.

Several published studies have investigated the population PK of pdVWF.^{17,24,31} However, these studies did not quantify the PK/PD relationship in a formal model. The current model-based analysis is in good agreement with the findings of an exploratory noncompartmental analysis,³² which was based on the observed VWF:RCo exposure data from patients with severe VWD who were administered rVWF in phase 1 (NCT00816660)¹⁹ and phase 3 on-demand (NCT01410227)⁶ studies. That exploratory noncompartmental analysis also showed longer MRT and $t_{1/2}$ values and a larger AUC_{0-inf} for rVWF versus pdVWF/FVIII with respect to VWF:RCo.³² A longer $t_{1/2}$ and larger AUC_{0-inf} of VWF:RCo following rVWF could potentially allow for lower doses and fewer infusions. In a recent multicenter, retrospective study conducted in France in patients with VWD who underwent surgery, a low number of infusions and low doses of rVWF (median [range] total dose: major surgery, 108 [22–340] rVWF IU/kg; minor surgery 37 [12–288] rVWF IU/kg) provided effective prevention of bleeding in major and minor surgeries.³³ Our results also suggest that rVWF can be administered without FVIII in most patients receiving surgery. In some cases where the FVIII:C level is <40 IU/dL, it may be necessary to administer a rFVIII product to achieve a hemostatic plasma level of FVIII:C.^{5,8}

The present results suggest a greater stabilization of endogenous FVIII due to slower elimination of VWF:RCo following rVWF administration compared with pdVWF/FVIII. This is in line with VWF acting as a chaperone for FVIII and protecting it from rapid clearance.¹ The longer FVIII $t_{1/2}$ following rVWF administration compared with pdVWF/FVIII might relate to differences in the VWF multimer profile of the two products. rVWF has a nonproteolytically degraded VWF multimer structure at administration and includes the most hemostatically active ultra-large multimers, which are not present in pdVWF/FVIII concentrates due to exposure to ADAMTS13 during manufacture.³⁴ pdVWF products contain a number of plasma-derived extraneous proteins, some including human albumin, that might lower their specific activity.³⁴

Because patients with type 3 VWD have a total or near-total absence of VWF, they are at an increased risk of bleeding, which can be life-threatening or lead to long-term complications.^{1,29} International management guidelines for VWD conditionally recommend long-term prophylaxis in patients with VWD and a history of severe and frequent bleeds.²⁹ We investigated VWF:RCo and FVIII:C profiles relevant to a prophylactic dosing regimen (ie, every 72 or 96 h). Simulations showed that following repeated administration of rVWF 50 IU/kg, FVIII:C >40 IU/dL can be maintained for the full 72 h between dosing, consistent with recommendations from the EMA.²⁵ Also based on the simulations in our study, a dosing schedule with an interval of 72 or 72/96 h could be a suitable option for prophylaxis in line with previous studies that demonstrate the potential for prophylaxis to help reduce bleed rates.^{35–38}

Several studies have demonstrated that repeated dosing of pdVWF/FVIII concentrates with a VWF:RCo/FVIII:C ratio of >1, results in less FVIII accumulation if VWF concentrate dosing is based only on VWF levels.^{39,40} However, FVIII accumulation has been observed after perioperative treatment with pdVWF/FVIII complex (human,

VWF:RCo/FVIII:C 2.4:1).⁴¹ In this work, simulations of the VWF:RCo and FVIII:C profiles following repeated administration of rVWF 50 IU/kg or pdVWF/FVIII 50 IU/kg (2.4:1 VWF/FVIII ratio) with either regular or irregular dosing intervals did not indicate marked FVIII accumulation. The aim of the simulations was to mimic VWF prophylaxis treatment regimens of approximately twice-weekly administration. More frequent dosing, such as during surgery, might increase the risk of factor VIII accumulation.⁴²

A study limitation was that the PK/PD comparison of rVWF and pdVWF/FVIII was based on a single pdVWF/FVIII (Humate-P, VWF:RCo/FVIII:C 2.4:1), so the study findings may not be applicable to other pdVWF concentrates with FVIII or to Wilfactin/Willfact (LFB, Les Ulis, France), which contains very little FVIII.⁴³ In addition, an *in silico* analysis of PK and PK/PD cannot fully reproduce *in vivo* interactions and due to the small number of patients in the current study, a formal covariate analysis was not possible for the pdVWF/FVIII PK model. However, given the mechanistic plausibility of the covariates identified for the rVWF model (body weight and hematocrit), these were applied also for the pdVWF/FVIII model.

Conclusion

The population PK and PK/PD models reported herein allowed for the *in silico* comparison of rVWF and pdVWF/FVIII with respect to the relationship between VWF:RCo and FVIII:C following administration of either product. The slower elimination of VWF:RCo following rVWF administration results in a prolonged effect on FVIII turnover compared with pdVWF/FVIII. The improved understanding of the PK and PK/PD of these products should help with the individualized treatment of patients with VWD based on PK/PD-guided dosing strategies.

Abbreviations

AUC₇₂, area under the activity–time curve during the dosing interval; C_{max}, maximum activity; FVIII, factor VIII; FVIII:C, FVIII activity; FVIII V, volume of distribution for FVIII; IC₅₀, activity level at half maximum inhibition; I_{max}, maximum inhibition; IIV, interindividual variability; LLOQ, lower limit of quantification; MRT, mean residence time; PD, pharmacodynamic; pdFVIII, plasma-derived FVIII; pdVWF, plasma-derived VWF; PK, pharmacokinetic; RCo, ristocetin cofactor; rFVIII, recombinant FVIII; rVWF, recombinant VWF; t_{1/2}, half-life; VWD, von Willebrand disease; VWF, von Willebrand factor.

Data Sharing Statement

The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants data supporting the results reported in this article, will be made available within three months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization. Requests should be sent to the corresponding author, Alexander Bauer.

Ethics Approval and Informed Consent

All trials contributing data for the research reported herein were approved by the respective institutional review boards or independent ethics committees at all participating sites ([Supplementary Table 2](#)), and patients provided written informed consent. The trials were conducted in accordance with the Declaration of Helsinki.

Acknowledgments

The authors acknowledge Bettina Ploder from Baxalta Innovations GmbH, a Takeda company, Vienna, Austria, who contributed to the conception and design of the study, and to the data collection and analysis of the data. The authors also acknowledge Björn Mellgard from Takeda Development Center Americas Inc., Cambridge, MA, USA, who contributed to the interpretation of the data, and Zhaoyang Li and Indranil Bhattacharya from Takeda Development Center Americas, Cambridge, USA, who provided expert review of the rVWF model data during the development of the manuscript. Medical writing support was provided by Nasser Malik, PhD, employee of Excel Medical Affairs (Fairfield, CT, USA), and was funded by Takeda Development Center Americas, Inc.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The study was funded by Baxalta Innovations GmbH, a Takeda company, Vienna, Austria, and Baxalta US Inc., a Takeda company, Lexington, MA, USA.

Disclosure

AB and MW are employees of Baxalta Innovations GmbH, a Takeda company, and are Takeda shareholders. SF-H is a co-owner and employee of Pharmetheus AB and GS is an employee of Pharmetheus AB. Both SF-H and GS have received funding from Baxalta Innovations GmbH and Baxalta US Inc for the conduct of this research. The authors report no other conflicts of interest in this work.

References

1. Leebeek FW, Eikenboom JC. Von Willebrand's disease. *N Engl J Med*. 2016;375(21):2067–2080. doi:10.1056/NEJMra1601561
2. Mannucci PM. Treatment of von Willebrand's disease. *N Engl J Med*. 2004;351(7):683–694. doi:10.1056/NEJMra040403
3. Budde U, Metzner HJ, Müller HG. Comparative analysis and classification of von Willebrand factor/factor VIII concentrates: impact on treatment of patients with von Willebrand disease. *Semin Thromb Hemost*. 2006;32(6):626–635. doi:10.1055/s-2006-949668
4. Reininger AJ. The function of ultra-large von Willebrand factor multimers in high shear flow controlled by ADAMTS13 [Die Funktion von ultragroßen von-Willebrand-Faktor-Multimeren und ihre Regulation durch ADAMTS 13 unter Flussbedingungen mit sehr hohen Scherraten]. *Hamostaseologie*. 2015;35(3):225–233. doi:10.5482/HAMO-14-12-0077
5. Baxalta US Inc. VONVENDI (von Willebrand factor [recombinant]) lyophilized powder for solution for intravenous injection. Available from: <https://www.fda.gov/media/94863/download>. Accessed September 26, 2022.
6. Gill JC, Castaman G, Windyga J, et al. Hemostatic efficacy, safety, and pharmacokinetics of a recombinant von Willebrand factor in severe von Willebrand disease. *Blood*. 2015;126(17):2038–2046. doi:10.1182/blood-2015-02-629873
7. Peyvandi F, Mamaev A, Wang JD, et al. Phase 3 study of recombinant von Willebrand factor in patients with severe von Willebrand disease who are undergoing elective surgery. *J Thromb Haemost*. 2019;17(1):52–62. doi:10.1111/jth.14313
8. Baxalta Innovations GmbH. VEYVONDI (vonicog alfa). Summary of product characteristics. Available from: https://www.ema.europa.eu/en/documents/product-information/veyvondi-epar-product-information_en.pdf. Accessed September 26, 2022.
9. Leebeek FW, Peyvandi F, Escobar MA, et al. Recombinant von Willebrand factor prophylaxis in patients with severe von Willebrand disease: phase 3 study results. *Blood*. 2022;140(2):89–98. doi:10.1182/blood.2021014810
10. Bello IF, Yuste VJ, Molina MQ, Navarro FH. Fanhdi, efficacy and safety in von Willebrand's disease: prospective international study results. *Haemophilia*. 2007;13(Suppl 5):25–32. doi:10.1111/j.1365-2516.2007.01570.x
11. Rivard GE, Aledort L. Alphanate surgical investigators. Efficacy of factor VIII/von Willebrand factor concentrate Alphanate in preventing excessive bleeding during surgery in subjects with von Willebrand disease. *Haemophilia*. 2008;14(2):271–275. doi:10.1111/j.1365-2516.2007.01616.x
12. Thompson AR, Gill JC, Ewenstein BM, Mueller-Velten G, Schwartz BA; Humate-P Study Group. Successful treatment for patients with von Willebrand disease undergoing urgent surgery using factor VIII/VWF concentrate (Humate-P). *Haemophilia*. 2004;10(1):42–51. doi:10.1046/j.1351-8216.2003.00809.x
13. Federici AB, Castaman G, Franchini M, et al. Clinical use of Haemate P in inherited von Willebrand's disease: a cohort study on 100 Italian patients. *Haematologica*. 2007;92(7):944–951. doi:10.3324/haematol.11124
14. Franchini M, Rossetti G, Tagliaferri A, et al. Efficacy and safety of factor VIII/von Willebrand's factor concentrate (Haemate-P) in preventing bleeding during surgery or invasive procedures in patients with von Willebrand disease. *Haematologica*. 2003;88(11):1279–1283.
15. Castaman G, Linari S. Vonicog alfa for the treatment of von Willebrand disease. *Expert Opin Orphan Drugs*. 2016;4(5):549–554. doi:10.1517/21678707.2016.1171138
16. Castaman G. Treatment of von Willebrand disease with FVIII/VWF concentrates. *Blood Transfus*. 2011;9(Suppl 2):s9–s13. doi:10.2450/2011.003S
17. Hazendonk HC, Heijdra JM, de Jager NCB, et al. Analysis of current perioperative management with Haemate[®] P/Humate P[®] in von Willebrand disease: identifying the need for personalized treatment. *Haemophilia*. 2018;24(03):460–470. doi:10.1111/hae.13451
18. Lethagen S, Kyrle PA, Castaman G, Haertel S, Mannucci PM; HAEMATE P Surgical Study Group. von Willebrand factor/factor VIII concentrate (Haemate P) dosing based on pharmacokinetics: a prospective multicenter trial in elective surgery. *J Thromb Haemost*. 2007;7(7):1420–1430. doi:10.1111/j.1538-7836.2007.02588.x
19. Mannucci PM, Kempton C, Millar C, et al. Pharmacokinetics and safety of a novel recombinant human von Willebrand factor manufactured with a plasma-free method: a prospective clinical trial. *Blood*. 2013;122(5):648–657. doi:10.1182/blood-2013-01-479527
20. European Medicines Agency. Guideline on reporting the results of population pharmacokinetic analyses. CHMP/EWP/185990/06. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-results-population-pharmacokinetic-analyses_en.pdf. Accessed November 5, 2020.

21. US Food and Drug Administration. Population pharmacokinetics. Guidance for industry. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/population-pharmacokinetics>. Accessed September 26, 2022.
22. Beal SL, Sheiner LB, Boeckmann AJ, Bauer RJ. *NONMEM Users' Guides (1989–2014)*. Icon Development Solutions; 2014.
23. R Foundation for Statistical Computing. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2019.
24. de Jager NCB, Bukkems LH, Heijdra JM, et al. One piece of the puzzle: population pharmacokinetics of FVIII during perioperative Haemate P[®]/Humate P[®] treatment in von Willebrand disease patients. *J Thromb Haemost*. 2020;18(2):295–305. doi:10.1111/jth.14652
25. European Medicines Agency. Guideline on the core SPC for human plasma derived von Willebrand factor (CPMP/BPWG/278/02). Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003423.pdf. Accessed October 5, 2020.
26. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia. *Haemophilia*. 2020;26(Suppl 6):1–158. doi:10.1111/hae.14046
27. Favaloro EJ. Towards personalised therapy for von Willebrand disease: a future role for recombinant products. *Blood Transfus*. 2016;14(2):262–276. doi:10.2450/2016.0258-15
28. Phua CW, Berntorp E. A personalized approach to the management of VWD. *Transfus Apher Sci*. 2019;58(5):590–595. doi:10.1016/j.transci.2019.08.009
29. Connell NT, Flood VH, Brignardello-Petersen R, et al. ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease. *Blood Adv*. 2021;5(1):301–325. doi:10.1182/bloodadvances.2020003264
30. Mansoor A, Mahabadi N. Volume of Distribution. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2021. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK545280/>. Accessed May 28, 2023.
31. Bukkems LH, Heijdra JM, de Jager NCB, et al. Population pharmacokinetics of the von Willebrand factor-factor VIII interaction in patients with von Willebrand disease. *Blood Adv*. 2021;5(5):1513–1522. doi:10.1182/bloodadvances.2020003891
32. European Medicines Agency. Veyvondi, summary of product characteristics and public assessment report. Available from: https://www.ema.europa.eu/en/documents/assessment-report/veyvondi-epar-public-assessment-report_en.pdf. Accessed May 28, 2023.
33. Desprez D, Drillaud N, Flaujac C, et al. Efficacy and safety of a recombinant von Willebrand factor treatment in patients with inherited von Willebrand disease requiring surgical procedures. *Haemophilia*. 2021;27(2):270–276. doi:10.1111/hae.14242
34. Turecek PL, Mitterer A, Matthiessen HP, et al. Development of a plasma- and albumin-free recombinant von Willebrand factor. *Hamostaseologie*. 2009;29(Suppl 1):S32–S38. doi:10.1055/s-0037-1617202
35. Abshire T, Cox-Gill J, Kempton CL, et al. Prophylaxis escalation in severe von Willebrand disease: a prospective study from the von Willebrand disease prophylaxis network. *J Thromb Haemost*. 2015;13(9):1585–1589. doi:10.1111/jth.12995
36. Abshire TC, Federici AB, Álvarez MT, et al. Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). *Haemophilia*. 2013;19(1):76–81. doi:10.1111/j.1365-2516.2012.02916.x
37. Berntorp E, Petrini P. Long-term prophylaxis in von Willebrand disease. *Blood Coagul Fibrinolysis*. 2005;16(Suppl 1):S23–S26. doi:10.1097/01.mbc.0000167659.23262.18
38. Holm E, Abshire TC, Bowen J, et al. Changes in bleeding patterns in von Willebrand disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network. *Blood Coagul Fibrinolysis*. 2015;26(4):383–388. doi:10.1097/MBC.0000000000000257
39. Raquet E, Stockschröder M, Dickneite G. Repeated infusions of VWF/FVIII concentrate: impact of VWF:FVIII ratio on FVIII trough and peak levels in a rabbit model. *Haemophilia*. 2011;17(5):808–814. doi:10.1111/j.1365-2516.2011.02603.x
40. Srivastava A, Serban M, Werner S, Schwartz BA, Kessler CM. Wonders Study Investigators. Efficacy and safety of a VWF/FVIII concentrate (wilate[®]) in inherited von Willebrand disease patients undergoing surgical procedures. *Haemophilia*. 2017;23(2):264–272. doi:10.1111/hae.13106
41. Gill JC, Shapiro A, Valentino LA, et al. von Willebrand factor/factor VIII concentrate (Humate-P) for management of elective surgery in adults and children with von Willebrand disease. *Haemophilia*. 2011;17(6):895–905. doi:10.1111/j.1365-2516.2011.02534.x
42. Peyvandi F, Kouides P, Turecek PL, Dow E, Berntorp E. Evolution of replacement therapy for von Willebrand disease: from plasma fraction to recombinant von Willebrand factor. *Blood Rev*. 2019;38:100572. doi:10.1016/j.blre.2019.04.001
43. Castaman G, Linari S. Human von Willebrand factor/factor VIII concentrates in the management of pediatric patients with von Willebrand disease/hemophilia A. *Ther Clin Risk Manag*. 2016;12:1029–1037. doi:10.2147/TCRM.S87543

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Pharmacologically Treated Anxiety and Depression in People Diagnosed with von Willebrand Disease: Matched Cohort Study

Katrina Wilcox Hagberg, Susan Jick, Gülden Özen & Ping Du

To cite this article: Katrina Wilcox Hagberg, Susan Jick, Gülden Özen & Ping Du (2023) Pharmacologically Treated Anxiety and Depression in People Diagnosed with von Willebrand Disease: Matched Cohort Study, Journal of Blood Medicine, , 413-425, DOI: [10.2147/JBM.S407993](https://doi.org/10.2147/JBM.S407993)

To link to this article: <https://doi.org/10.2147/JBM.S407993>



© 2023 The Takeda Pharmaceutical Company Limited.



Published online: 11 Jul 2023.



Submit your article to this journal



Article views: 260



View related articles



View Crossmark data

Pharmacologically Treated Anxiety and Depression in People Diagnosed with von Willebrand Disease: Matched Cohort Study

Katrina Wilcox Hagberg¹, Susan Jick^{1,2}, Gülden Özen³, Ping Du³

¹Boston Collaborative Drug Surveillance Program, Lexington, MA, 02421, USA; ²Boston University School of Public Health, Boston, MA, 02118, USA; ³Takeda Development Center Americas, Inc., Cambridge, MA, 02139, USA

Correspondence: Susan Jick, Boston Collaborative Drug Surveillance Program, 11 Muzzey Street, Lexington, MA, 02421, USA, Tel +1 781 862 6660, Fax +1 781 862 1680, Email sjick@bu.edu

Background: People diagnosed with von Willebrand disease (VWD) have reduced quality of life versus the general population, and there is limited evidence of increased rates of anxiety and/or depression among people diagnosed with VWD.

Aim: To understand the association between VWD and mental health outcomes.

Design and Setting: A retrospective cohort study was conducted using the UK Clinical Practice Research Datalink (CPRD) GOLD database (1988–2016).

Methods: People diagnosed with VWD were matched 1:10 to randomly selected people in the database without VWD based on sex, birth year ± 2 years, CPRD record start year ± 2 years, and general practice attended. Individuals were followed from VWD diagnosis or match date to censoring (first event date, CPRD end date, or death). Treated anxiety and treated depression were identified by a diagnostic Read Code and a prescription for anxiety/depression medication recorded within 90 days of each other, after VWD diagnosis/match date.

Results: Treated anxiety was recorded in 89 of 1119 (8.0%) people diagnosed with VWD and 624 of 10,423 (6.0%) without VWD (age- and sex-adjusted incidence rate ratio [IRR], 1.37; 95% confidence interval [CI], 1.10–1.71). Treated depression was recorded in 119 of 1083 (11.0%) people diagnosed with VWD and 846 of 9845 (8.6%) without VWD (adjusted IRR, 1.35; 95% CI, 1.11–1.63). Females aged 20–39 and 0–19 years were at greatest risk for treated anxiety and treated depression, respectively.

Conclusion: Higher rates of treated anxiety and depression were observed among people diagnosed with versus without VWD, predominantly in young females.

Keywords: anxiety, depression, general practice, mental health, von Willebrand disease

Introduction

von Willebrand factor is a clotting protein that mediates platelet functions and stabilizes clotting factor VIII.¹ Congenital von Willebrand disease (VWD) is characterized by impaired function or reduced levels of von Willebrand factor, and is the most common inherited bleeding disorder globally (prevalence of 600–1300 per 100,000 individuals).² The prevalence of symptomatic disease requiring treatment is, however, much lower (10 per 100,000). VWD bleeding phenotypes vary depending on disease type, age, and sex.³ Common symptoms include bruising, epistaxis, bleeding from wounds or surgeries, joint and mucocutaneous bleeding, and menorrhagia.^{3–5} Rare and potentially life-threatening bleeding can occur in the central nervous system and gastrointestinal tract.^{3,5}

People diagnosed with VWD have lower health-related quality of life, as measured by Short Form 36 mental component scores, than the general population,^{6,7} with some evidence suggesting that depression and anxiety are more common in people diagnosed with VWD.⁸ Bleeding disorders (hemophilia and VWD) have been reported to have a negative impact on psychological health, with unpredictable bleeding symptoms and fears for the future cited as causes of anxiety, and loss of or reduction in valued activities and loss of independence as causes of depression.⁹ For example,

women diagnosed with VWD experience impaired quality of life and greater impact on daily activities as a result of menstruation than individuals without VWD.¹⁰ In addition, misdiagnosis and delayed diagnosis of VWD are common, particularly in women, and lead to worse outcomes,^{11–13} which may in turn contribute to impaired quality of life and mental health. Data on mental health disorders in people diagnosed with VWD are limited, however, and more information is needed to improve their overall health and wellbeing. This retrospective cohort study was conducted using UK clinical practice data to better understand the occurrence of treated anxiety and treated depression in people diagnosed with VWD.

Methods

Data Source

Data were extracted from the UK Clinical Practice Research Datalink (CPRD) GOLD database (January 1, 1988 to June 30, 2016), which is a large, prospectively collected, anonymized electronic medical record database that is broadly representative of the UK population, in terms of age, sex, and minority distribution. As UK health care is universal and free, selection bias resulting from differential health care access is minimized. CPRD GOLD comprises demographic information, prescription details, clinical events, and lifestyle details. Information relating to inpatient and outpatient medical encounters (specialists, hospital stays) is provided to individuals' general practitioners (GPs) in consultant and discharge letters, and GPs may code these details in the patient's electronic data. The study population was from the general practice setting and therefore is representative of individuals receiving general practice care.¹⁴

Study Population

Individuals with a Read Code indicating a diagnosis of VWD (D304.00), recorded at any time, were included. Individuals were excluded if they were suspected of having acquired von Willebrand syndrome due to a diagnosis of certain autoimmune disorders (ie, lymphoproliferative diseases, monoclonal gammopathies, systemic lupus erythematosus, hypothyroidism, essential thrombocythemia, polycythemia vera, myelofibrosis) or cancers (eg, myeloproliferative neoplasms).¹⁴ All other individuals were assumed to have congenital VWD, regardless of age at first recorded VWD diagnosis.

Each person with VWD was matched to up to 10 people without VWD, selected randomly from all people in the database without a diagnosis of VWD. Matching criteria were sex, birth year (± 2 years), CPRD record start year (± 2 years), and general practice attended. As the case definitions for anxiety and depression required a diagnosis plus pharmaceutical treatment to be recorded in the electronic medical record, the study population was restricted to individuals whose first VWD diagnosis or match date was recorded after the start of the electronic CPRD record.

Outcomes of Interest

Treated anxiety or treated depression events were identified by a diagnostic Read Code for anxiety and/or depression plus receipt of a prescription for drugs used to treat anxiety or depression ([Supplemental Table 1](#)) within 90 days of each other, both recorded in the electronic record after the VWD diagnosis or match date. The index date was defined as the date of the qualifying diagnosis or medication prescription, whichever came first. To exclude pre-existing anxiety or depression, individuals with prior treated anxiety or treated depression events before the VWD diagnosis or match date were excluded from the relevant analyses.

Eligible individuals were followed from the VWD diagnosis or match date until the censoring date, defined as the index date, date of electronic record end, or death, whichever occurred first.

Covariates

In addition to the matching criteria, the presence of covariates related to general health at the VWD diagnosis/match date (including events present in the historical record) and at the case index date were described: hypertension, diabetes, hypercholesterolemia, chronic kidney disease, liver disease, alcohol abuse, and drug abuse. The presence of an anxiety or depression diagnosis before the VWD diagnosis/match date was also evaluated.

Statistical Analysis

Treated anxiety and treated depression were evaluated separately. Demographics were described at the VWD diagnosis/match date for the VWD and matched non-VWD cohorts, and at the index date for individuals with treated depression and/or anxiety. Person-years (PY) were accumulated separately for each outcome from the VWD diagnosis/match date to the censor date (ie, the index date for treated anxiety or depression, date of Clinical Practice Research Datalink record end, or death, whichever occurred first).

Incidence rates (IRs) with 95% confidence intervals (CIs) were calculated for each outcome for participants diagnosed with and without VWD overall and stratified by sex and age at treated anxiety or depression date using Byar's method. Crude, sex- and age-adjusted incidence rate ratios (IRRs) with 95% CIs for each outcome for people diagnosed with VWD versus those without VWD were calculated using Poisson regression. The risk (cumulative incidence function) was also estimated for each outcome using the Kaplan–Meier method, overall and stratified by age at VWD diagnosis/match date. Kaplan–Meier curves were compared using a Log rank test. Descriptive and IR analyses were performed using SAS (version 9.4). All Kaplan–Meier and Cox model analyses were performed using R (version 4.0.2).

Results

Treated Anxiety

The baseline characteristics of 1119 people diagnosed with VWD and 10,423 matched individuals without VWD included in the anxiety analysis are shown in Table 1. The majority of people were female (62.8% with VWD; 62.4% without VWD), and most were aged ≤30 years at VWD diagnosis/match date (61.2% with VWD; 62.3% without VWD). A diagnosis of anxiety (without an associated prescription) was recorded before VWD diagnosis in 6.3% of people diagnosed with VWD and 3.9% of those without VWD.

Table 1 Baseline Characteristics of Individuals Included in the Treated Anxiety Analysis and Characteristics of Treated Anxiety Cases

	All Individuals in the Treated Anxiety Analysis		Individuals Diagnosed with Treated Anxiety After Cohort Entry (Cases)	
	Individuals with VWD (n = 1119)	Individuals without VWD (n = 10,423)	Individuals with VWD (n = 89)	Individuals without VWD (n = 624)
Sex				
Female	703 (62.8)	6508 (62.4)	75 (84.3)	524 (84.0)
Male	416 (37.2)	3915 (37.6)	14 (15.7)	100 (16.0)
Year of first VWD diagnosis/match date				
1988–1994	91 (8.1)	841 (8.1)	–	–
1995–1999	219 (19.6)	2079 (20.0)	–	–
2000–2004	262 (23.4)	2459 (23.6)	–	–
2005–2009	301 (26.9)	2761 (26.5)	–	–
2010–2016	246 (22.0)	2283 (21.9)	–	–
Age, years	At first VWD diagnosis/match date		At treated anxiety date	
Mean ± SD	25.7 ± 19.4	25.2 ± 19.2	39.5 ± 16.7	38.9 ± 15.6
Median (range)	21 (0–87)	21 (0–86)	35 (14–76)	37 (13–91)

(Continued)

Table 1 (Continued).

	All Individuals in the Treated Anxiety Analysis		Individuals Diagnosed with Treated Anxiety After Cohort Entry (Cases)	
	Individuals with VWD (n = 1119)	Individuals without VWD (n = 10,423)	Individuals with VWD (n = 89)	Individuals without VWD (n = 624)
Age category, years				
<10	292 (26.1)	2811 (27.1)	0 (0.0)	0 (0.0)
10–19	223 (19.9)	2124 (20.4)	7 (7.9)	47 (7.5)
20–29	170 (15.2)	1543 (14.8)	26 (29.2)	168 (26.9)
30–39	160 (14.3)	1451 (13.9)	16 (18.0)	117 (18.8)
40–49	128 (11.4)	1165 (11.2)	10 (11.2)	141 (22.6)
50–59	72 (6.4)	691 (6.6)	17 (19.1)	86 (13.8)
60–69	45 (4.0)	395 (3.8)	10 (11.2)	37 (5.9)
≥70	29 (2.6)	243 (2.3)	NR	28 (4.5)
Treated anxiety diagnosis year				
1988–1994	–	–	NR	5 (0.8)
1995–1999	–	–	NR	34 (5.5)
2000–2004	–	–	15 (16.9)	115 (18.4)
2005–2009	–	–	23 (25.8)	159 (25.5)
2010–2016	–	–	47 (52.8)	311 (49.8)
Length of follow-up (VWD diagnosis/match date to censor date ^a), years				
Mean ± SD	7.2 ± 5.7	7.5 ± 5.8	–	–
Median (range)	6.0 (0.1–27.1)	6.3 (0.1–27.4)		
Length of follow-up category, years				
<1	122 (10.9)	1094 (10.5)	–	–
1–2.9	203 (18.1)	1720 (16.5)	–	–
3–4.9	148 (13.2)	1456 (14.0)	–	–
≥5	646 (57.7)	6153 (59.0)	–	–
Covariates	Before VWD diagnosis/match date		Before treated anxiety diagnosis date	
Anxiety diagnosis ^b	70 (6.3)	401 (3.9)	41 (46.1)	271 (43.4)
Prescription for anxiolytics ^c	191 (17.1)	1286 (12.3)	71 (78.8)	449 (72.0)
Hypertension	66 (5.9)	419 (4.0)	14 (15.7)	74 (11.9)
Diabetes	15 (1.3)	142 (1.4)	NR	21 (3.4)
Hypercholesterolemia	15 (1.3)	181 (1.7)	5 (5.6)	32 (5.1)
Chronic kidney disease	NR	46 (0.4)	NR	9 (1.4)
Liver disease	NR	10 (0.1)	0 (0.0)	5 (0.8)

(Continued)

Table 1 (Continued).

	All Individuals in the Treated Anxiety Analysis		Individuals Diagnosed with Treated Anxiety After Cohort Entry (Cases)	
	Individuals with VWD (n = 1119)	Individuals without VWD (n = 10,423)	Individuals with VWD (n = 89)	Individuals without VWD (n = 624)
Alcohol abuse	27 (2.4)	267 (2.6)	5 (5.6)	49 (7.9)
Drug abuse	5 (0.5)	62 (0.6)	NR	17 (2.7)
Reason for censor				
End of record	1010 (90.3)	9619 (92.3)	–	–
Treated anxiety case	89 (8.0)	624 (6.0)	–	–
Death	20 (1.8)	180 (1.7)	–	–

Notes: Data are presented as n (%) unless otherwise stated. ^aCensor date defined as the date the patient became a case, date of record end, or death, whichever occurred first. ^bAnxiety diagnosis without a prescription for anxiolytic within 90 days of each other (not recorded as a treated anxiety event). ^cPrescription for anxiolytics without an anxiety diagnosis within 90 days of each other (not recorded as a treated anxiety event). It is possible that the anxiolytic prescription was for another indication for use. **Abbreviations:** NR, not reportable (counts of 1–5 individuals); SD, standard deviation; VWD, von Willebrand disease.

Treated anxiety was recorded in 89 people with VWD (8.0%) and 624 without VWD (6.0%) (Table 1). People diagnosed with VWD and treated anxiety were slightly younger than those without VWD (median age of 35.0 versus 37.0 years, respectively). Similar numbers of individuals in the VWD and non-VWD cohorts had a record of an anxiety diagnosis without a prescription or a prescription without a diagnosis before they met the criteria for treated anxiety. The number of individuals with other medical conditions was low and relatively balanced between the two cohorts.

The cumulative incidence of treated anxiety was higher in people diagnosed with VWD versus those without VWD ($P = 0.0038$) (Figure 1). In stratified analyses, cumulative incidence was higher in females diagnosed with VWD versus those without VWD ($P = 0.011$), but not in males ($P = 0.22$) (Supplemental Figure 1), and was higher for people with versus without VWD for all age groups, but was only statistically significant for individuals aged <10 years ($P = 0.042$) or 20–29

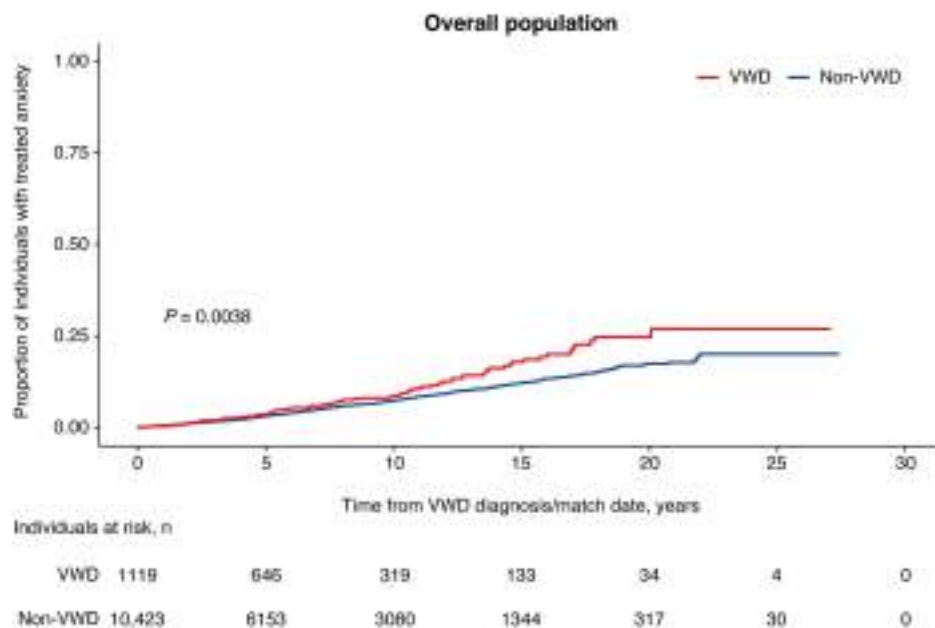


Figure 1 Cumulative incidence of treated anxiety after VWD diagnosis/match date among individuals with versus without VWD.

Abbreviation: VWD, von Willebrand disease.

years ($P = 0.033$) (Supplemental Figure 2). When stratified by sex and age, cumulative incidence was only statistically significant for females aged 20–29 years at VWD diagnosis or match date ($P = 0.012$) (Supplemental Figure 3).

The IR for treated anxiety was 11.0 (95% CI, 8.8–13.5) per 1000 PY for people diagnosed with VWD and 8.0 (95% CI, 7.3–8.6) per 1000 PY for people without VWD (Table 2). The IR for treated anxiety among females was 14.8 (95% CI, 11.6–18.5) per 1000 PY for those diagnosed with VWD and 10.9 (9.9–11.8) per 1000 PY for non-VWD matches, whereas the IRs for males were lower (4.6 [95% CI, 2.5–7.8] and 3.3 [95% CI, 2.7–4.0] per 1000 PY, respectively). IRs for treated anxiety varied by age and by age and sex (Table 2).

Table 2 IRs and IRRs of Treated Anxiety in Individuals with or without VWD

	Individuals with VWD		Individuals without VWD (Reference)		IRRs ^a	
	Treated Anxiety Cases (PY)	IR per 1000 PY (95% CI)	Treated Anxiety Cases (PY)	IR per 1000 PY (95% CI)	Crude IRR ^a (95% CI)	Adjusted IRR ^a (95% CI)
Overall	89 (8110)	11.0 (8.8–13.5)	624 (78,409)	8.0 (7.3–8.6)	1.38 (1.10–1.72)	1.37 (1.10–1.71) ^b
By sex						
Female	75 (5079)	14.8 (11.6–18.5)	524 (48,293)	10.9 (9.9–11.8)	1.36 (1.07–1.73)	1.36 (1.07–1.74) ^c
Male	14 (3031)	4.6 (2.5–7.8)	100 (30,116)	3.3 (2.7–4.0)	1.39 (0.80–2.43)	1.39 (0.79–2.43) ^c
By age group, years ^d						
0–19	7 (2873)	2.4 (1.0–5.0)	47 (28,375)	1.7 (1.2–2.2)	1.47 (0.66–3.25)	1.45 (0.65–3.20) ^e
20–39	42 (2247)	18.7 (13.5–25.3)	285 (23,193)	12.3 (10.9–13.8)	1.52 (1.10–2.10)	1.51 (1.09–2.08) ^e
40–59	27 (2146)	12.6 (8.3–18.3)	227 (18,883)	12.0 (10.5–13.7)	1.05 (0.70–1.56)	1.05 (0.70–1.57) ^e
≥60	13 (844)	15.4 (8.2–26.3)	65 (7957)	8.2 (6.3–10.4)	1.89 (1.04–3.42)	1.88 (1.03–3.40) ^e
By sex and age group, years ^d						
Female						
0–19	5 (1427)	3.5 (1.1–8.2)	40 (13,745)	2.9 (2.1–4.0)	1.20 (0.48–3.05)	–
20–39	37 (1542)	24.0 (16.9–33.1)	233 (15,615)	14.9 (13.1–17.0)	1.61 (1.14–2.27)	–
40–59	25 (1548)	16.1 (10.4–23.8)	197 (13,707)	14.4 (12.4–16.5)	1.12 (0.74–1.70)	–
≥60	8 (562)	14.2 (6.1–28.1)	54 (5226)	10.3 (7.8–13.5)	1.38 (0.66–2.90)	–
Male						
0–19	NR (1446)	1.4 (0.2–5.0)	7 (14,630)	0.5 (0.2–1.0)	2.89 (0.60–13.92)	–
20–39	5 (705)	7.1 (2.3–16.6)	52 (7579)	6.9 (5.1–9.0)	1.03 (0.41–2.59)	–
40–59	NR (598)	3.3 (0.4–12.1)	30 (5176)	5.8 (3.9–8.3)	0.58 (0.14–2.42)	–
≥60	5 (282)	17.7 (5.7–41.3)	11 (2731)	4.0 (2.0–7.2)	4.40 (1.53–12.66)	–

Notes: ^aIRR of treated anxiety for individuals with VWD compared with those without VWD (reference). ^bIRR adjusted for sex and age. ^cIRR adjusted for age. ^dBased on age at treated anxiety date. ^eIRR adjusted for sex.

Abbreviations: CI, confidence interval; IR, incidence rate; IRR, incidence rate ratio; NR, not reportable (counts of 1–5 individuals); PY, person-years; VWD, von Willebrand disease.

Overall, the risk of treated anxiety was higher for people diagnosed with VWD than those without VWD (age- and sex-adjusted IRR, 1.37 [95% CI, 1.10–1.71]). Risk estimates were similar among females and males but varied by age, with a higher risk observed in individuals aged 20–39 years or ≥60 years (Table 2). When stratified by sex and age, the greatest risk of treated anxiety in females was observed among those aged 20–39 years, with these individuals having a 61% higher rate of treated anxiety than those without VWD. There were too few cases for risk estimates for males to be reliable when stratified by age group.

Treated Depression

Baseline characteristics for the 1083 people diagnosed with VWD and 9845 matched individuals without VWD included in the treated depression analysis are shown in Table 3. The majority of people were female (62.1% with VWD; 61.2% without VWD) and most (61.9% diagnosed with VWD; 63.8% without VWD) were aged ≤ 30 years at VWD diagnosis/match date. A diagnosis of prior non-treated depression was recorded in 7.2% of people with VWD and 4.9% of those without VWD.

Table 3 Baseline Characteristics of Individuals Included in the Treated Depression Analysis and Characteristics of Treated Depression Cases

	All Individuals in the Treated Depression Analysis		Individuals Diagnosed with Treated Depression After Cohort Entry (Cases)	
	Individuals Diagnosed with VWD (n = 1083)	Individuals without VWD (n = 9845)	Individuals Diagnosed with VWD (n = 119)	Individuals without VWD (n = 846)
Sex				
Female	673 (62.1)	6022 (61.2)	104 (87.4)	695 (82.2)
Male	410 (37.9)	3823 (38.8)	15 (12.6)	151 (17.9)
Year of first VWD diagnosis/match date				
1988–1994	89 (8.2)	817 (8.3)	–	–
1995–1999	216 (19.9)	2021 (20.5)	–	–
2000–2004	257 (23.7)	2334 (23.7)	–	–
2005–2009	283 (26.1)	2509 (25.5)	–	–
2010–2016	238 (22.0)	2164 (22.0)	–	–
Age, years	At first VWD diagnosis/match date		At treated depression date	
Mean $\pm$ SD	25.3 $\pm$ 19.5	24.4 $\pm$ 19.2	37.6 $\pm$ 14.6	38.1 $\pm$ 14.5
Median (range)	21 (0–86)	19 (0–86)	35 (15–88)	37 (15–88)
Age category, years				
<10	292 (27.0)	2810 (28.5)	0 (0.0)	0 (0.0)
10–19	222 (20.5)	2113 (21.5)	12 (10.1)	59 (7.0)
20–29	156 (14.4)	1360 (13.8)	32 (26.9)	240 (28.4)
30–39	151 (14.0)	1320 (13.4)	20 (16.8)	168 (19.9)
40–49	123 (11.4)	1059 (10.8)	28 (23.5)	182 (21.5)
50–59	66 (6.1)	582 (5.9)	19 (16.0)	138 (16.3)
60–69	45 (4.2)	371 (3.8)	5 (4.2)	37 (4.4)
≥ 70	28 (2.6)	230 (2.3)	NR	22 (2.6)
Treated depression diagnosis year				
1988–1994	–	–	NR	13 (1.5)
1995–1999	–	–	8 (6.7)	72 (8.5)
2000–2004	–	–	29 (24.4)	210 (24.8)
2005–2009	–	–	37 (31.1)	220 (26.0)
2010–2016	–	–	44 (25.0)	331 (39.1)

(Continued)

Table 3 (Continued).

	All Individuals in the Treated Depression Analysis		Individuals Diagnosed with Treated Depression After Cohort Entry (Cases)	
	Individuals Diagnosed with VWD (n = 1083)	Individuals without VWD (n = 9845)	Individuals Diagnosed with VWD (n = 119)	Individuals without VWD (n = 846)
Length of follow-up (VWD diagnosis/match date to censor date ^a)				
Mean ± SD	7.0 ± 5.7	7.4 ± 5.8	–	–
Median (range)	5.7 (0.1–27.1)	6.1 (0.1–27.4)		
Length of follow-up category, years				
<1	131 (12.1)	1084 (11.0)	–	–
1–2.9	197 (18.2)	1667 (16.9)	–	–
3–4.9	149 (13.8)	1426 (14.5)	–	–
≥5	606 (56.0)	5668 (57.6)	–	–
Covariates	Before VWD diagnosis/match date		Before treated depression date	
Depression diagnosis ^b	78 (7.2)	486 (4.9)	56 (47.1)	444 (52.5)
Prescription for antidepressant ^c	110 (10.2)	705 (7.2)	84 (70.6)	551 (65.1)
Hypertension	62 (5.7)	384 (3.9)	10 (8.4)	79 (9.3)
Diabetes	15 (1.4)	130 (1.3)	NR	23 (2.7)
Hypercholesterolemia	17 (1.6)	164 (1.7)	NR	38 (4.5)
Chronic kidney disease	NR	45 (0.5)	NR	13 (1.5)
Liver disease	NR	9 (0.1)	0 (0.0)	NR
Alcohol abuse	23 (2.1)	229 (2.3)	8 (6.7)	60 (7.1)
Drug abuse	NR	48 (0.5)	NR	20 (2.4)
Reason for censor				
End of record	943 (87.1)	8848 (90.0)	–	–
Treated anxiety case	119 (11.0)	846 (8.6)	–	–
Death	21 (1.9)	151 (1.5)	–	–

Notes: Data are presented as n (%) unless otherwise stated. ^aCensor date defined as the date the patient became a case, date of record end, or death, whichever occurred first.

^bDepression diagnosis without a prescription for antidepressant within 90 days of each other (not recorded as a treated depression event). ^cPrescription for antidepressant without a depression diagnosis within 90 days of each other (not recorded as a treated depression event). It is possible that the antidepressant prescription was for another indication for use.

Abbreviations: NR, not reportable (counts 1–5 individuals); SD, standard deviation; VWD, von Willebrand disease.

Treated depression was recorded in 119 people (11.0%) diagnosed with VWD and 846 without VWD (8.6%) (Table 3). People diagnosed with VWD were slightly younger than those without VWD (median age of 35.0 versus 37.0 years, respectively). In the VWD and non-VWD cohorts, similar numbers of treated depression cases had a record of a depression diagnosis without a prescription or a prescription without a diagnosis before the individual met the criteria to be a treated depression case. The number of individuals with other conditions related to general health was low and similar between cohorts.

The cumulative incidence of treated depression was statistically significantly higher in people diagnosed with VWD than in those without VWD ($P = 0.0024$) (Figure 2). When stratified by sex and/or age at VWD diagnosis/match date, the cumulative incidence was statistically significantly higher in females with versus without VWD ($P = 0.0012$), individuals of both sexes aged 20–29 years ($P = 0.0089$), females aged <10 years ($P = 0.047$), or females aged 20–29 years ($P = 0.0023$) (Supplemental Figures 4–6).

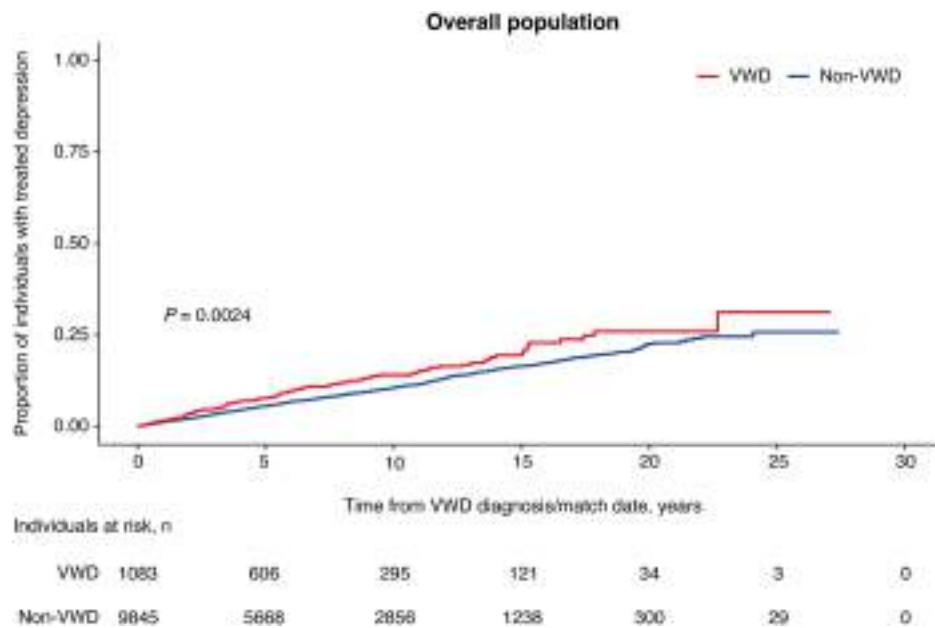


Figure 2 Cumulative incidence of treated depression after VWD diagnosis/match date among individuals with versus without VWD.

Abbreviation: VWD, von Willebrand disease.

The IR for treated depression was 15.6 (95% CI, 12.9–18.7) per 1000 PY for people diagnosed with VWD and 11.6 (95% CI, 10.8–12.4) per 1000 PY for those without VWD (Table 4). Among females, the IR for treated depression was 22.3 (95% CI, 18.2–27.0) per 1000 PY for the VWD and 15.9 (95% CI, 14.7–17.1) per 1000 PY for the non-VWD cohorts, whereas the IRs for males were lower (5.1 [95% CI, 2.8–8.3] and 5.2 [95% CI, 4.4–6.1]) per 1000 PY, respectively). IRs for treated depression were highest between the ages of 20 and 59 years, and varied when stratified by age and sex (Table 4).

Table 4 IRs and IRRs of Treated Depression in Individuals with or without VWD

	Individuals with VWD		Individuals without VWD (Reference)		IRRs ^a	
	Treated Depression Cases (PY)	IR per 1000 PY (95% CI)	Treated Depression Cases (PY)	IR per 1000 PY (95% CI)	Crude IRR ^a (95% CI)	Adjusted IRR ^a (95% CI)
Overall	119 (7634)	15.6 (12.9–18.7)	846 (72,922)	11.6 (10.8–12.4)	1.34 (1.11–1.63)	1.35 (1.11–1.63) ^b
By sex						
Female	104 (4666)	22.3 (18.2–27.0)	695 (43,764)	15.9 (14.7–17.1)	1.40 (1.14–1.72)	1.40 (1.14–1.72) ^c
Male	15 (2968)	5.1 (2.8–8.3)	151 (29,158)	5.2 (4.4–6.1)	0.98 (0.57–1.66)	0.96 (0.56–1.63) ^c
By age group, years ^d						
0–19	12 (2862)	4.2 (2.2–7.3)	59 (28,326)	2.1 (1.6–2.7)	2.01 (1.08–3.74)	1.98 (1.07–3.69) ^e
20–39	52 (2016)	25.8 (19.3–33.8)	408 (20,482)	19.9 (18.0–22.0)	1.30 (0.97–1.73)	1.29 (0.96–1.72) ^e
40–59	47 (1931)	24.3 (17.9–32.4)	320 (16,647)	19.2 (17.2–21.4)	1.27 (0.93–1.72)	1.26 (0.47–1.71) ^e
≥60	8 (825)	9.7 (4.2–19.1)	59 (7468)	7.9 (6.0–10.2)	1.23 (0.59–2.57)	1.23 (0.59–2.58) ^e

(Continued)

Table 4 (Continued).

	Individuals with VWD		Individuals without VWD (Reference)		IRRs ^a	
	Treated Depression Cases (PY)	IR per 1000 PY (95% CI)	Treated Depression Cases (PY)	IR per 1000 PY (95% CI)	Crude IRR ^a (95% CI)	Adjusted IRR ^a (95% CI)
By sex and age group, years ^d						
Female						
0–19	10 (1412)	7.1 (3.4–13.0)	51 (13,698)	3.7 (2.8–4.9)	1.90 (0.97–3.75)	–
20–39	47 (1313)	35.8 (26.3–47.6)	333 (13,168)	25.3 (22.6–28.2)	1.42 (1.17–1.92)	–
40–59	43 (1399)	30.7 (22.2–41.4)	266 (11,908)	22.3 (19.7–25.2)	1.38 (1.00–1.90)	–
≥60	NR (541)	7.4 (2.0–18.9)	45 (4989)	9.0 (6.6–12.1)	0.82 (0.29–2.28)	–
Male						
0–19	NR (1449)	1.4 (0.2–5.0)	8 (14,627)	0.5 (0.2–1.1)	2.52 (0.54–11.88)	–
20–39	5 (703)	7.1 (2.3–16.6)	75 (7314)	10.3 (8.1–12.9)	0.69 (0.28–1.72)	–
40–59	NR (532)	7.5 (2.0–19.3)	54 (4738)	11.4 (8.6–14.9)	0.66 (0.24–1.82)	–
≥60	NR (284)	14.1 (3.8–36.0)	14 (2479)	5.6 (3.1–9.5)	2.49 (0.82–7.57)	–

Notes: ^aIRR of treated depression for individuals with VWD compared to those without VWD (reference). ^bIRR adjusted for sex and age. ^cIRR adjusted for age. ^dBased on age at treated depression date. ^eIRR adjusted for sex.

Abbreviations: CI, confidence interval; IR, incidence rate; IRR, incidence rate ratio; NR, not reportable (counts of 1–5 individuals); PY, person-years; VWD, von Willebrand disease.

The risk of treated depression was higher for people diagnosed with VWD than for those without VWD (age- and sex-adjusted IRR, 1.35 [95% CI, 1.11–1.63]) (Table 4). IRRs remained higher in females with versus without VWD when adjusted for age at treated depression date (IRR, 1.40 [95% CI, 1.14–1.72]). For males, however, the age-adjusted risk of treated depression was not higher in individuals with VWD than in those without VWD (IRR, 0.96 [95% CI, 0.56–1.63]). The IRR was highest for people (of both sexes) diagnosed with VWD aged 0–19 years compared with those without VWD when stratified by age group (Table 4). When stratified by age and sex, the greatest risk was observed in females aged 0–19 years at treated depression date and was also elevated for females aged 20–39 and 40–59, compared to similarly aged females without VWD. IRs and IRRs for males were based on too few cases to be informative.

Discussion

This retrospective cohort study describes mental health-related outcomes in people diagnosed with VWD in UK general practice. Overall, treated anxiety and treated depression were more common in individuals with VWD than in matched individuals without VWD. Females aged 20–39 and 0–19 years were at greatest risk for treated anxiety and treated depression, respectively.

Anxiety and depression rates in our study were substantially less than those in a prospective study in individuals attending hemophilia treatment centers in the USA.⁸ Overall rates of anxiety and depression in the US study were 58% and 64%, respectively, versus 8% and 11% among people diagnosed with VWD managed by GPs in this UK-based study. VWD complications associated with depression in the US study included self-reported chronic pain and joint problems, while joint problems were also associated with anxiety.⁸ In addition, findings revealed that joint problems and not being in a relationship (ie, single, widowed, or divorced) were important predictors of depression and anxiety, while anxiety was also more common in adolescents (12–18 years of age) and individuals whose general health had deteriorated in the prior 3 months.⁸ Although the study populations differed between the two studies, higher rates of anxiety and depression

were observed in females diagnosed with VWD compared with males in both studies. The definitions of anxiety and depression in our study were more restrictive than those in the US study, in which diagnosis was based on patient-reported outcomes at the last follow-up visit (7-item Generalized Anxiety Disorder questionnaire and 8-item Patient Health Questionnaire).⁸ The case definition in our study required a formal diagnosis plus a prescription for anxiety or depression treatment within 90 days. This definition was intended to capture clinically relevant anxiety or depression requiring GP treatment, which should have been reliably recorded in the UK CPRD database.^{15–17} Thus, the incidence of anxiety and treated depression may have been underestimated in our study because only clinically treated individuals were identified. Also, individuals in whom diagnosis and treatment were recorded >90 days apart, or in whom diagnosis and treatment took place outside of primary care, would not appear in the analysis. However, this misclassification should not differ between the VWD and non-VWD populations, as individuals were matched on general practice, leading to similar GP recording habits.

This was a population-based study using data from the UK, where universal health care results in few barriers to care. All people diagnosed with VWD covered in CPRD GOLD who had a VWD diagnosis at any time were, therefore, captured. This is an important strength of our analysis, as the study cohort is likely to include individuals with a wider range of VWD severity than those being cared for by specialty centers and is likely to be representative of individuals seeking general practice care. This is in contrast to previous studies of quality of life and mental health in people diagnosed with VWD, which usually focus on those in specialist care.^{6–8} Another strength of this study is that individuals without VWD were matched to people diagnosed with VWD based on sex, age, calendar year, and GP attended, to control for differences in risk and data availability by these matching factors. A limitation of the study is that electronic medical records are not available for care provided in secondary/specialist care settings. Although GPs receive and have access to letters from specialists detailing care provided in secondary care settings, the GP may not have recorded these in a way that is accessible to researchers. In addition, detailed information about VWD type and severity is not available in the CPRD database. The small number of males diagnosed with VWD in the analysis meant that reliable estimates could not be calculated for this population. Finally, cardiac valvular disease, which is an underlying disease for acquired von Willebrand syndrome typically in elderly populations,¹⁸ was not included as an exclusion criteria. However, only 2.6% of individuals with VWD and 2.3% of those without VWD in the study population were aged ≥ 70 years at the VWD diagnosis/match date. Therefore, the potential inclusion of elderly patients with acquired von Willebrand syndrome due to cardiac valvular disease is likely to have minimal impact on the results of this study.

Conclusions

In this retrospective cohort study, treated anxiety and treated depression were more common in people diagnosed with VWD than in those without VWD, predominantly in females. These findings highlight mental health-related conditions as a potential unmet need in people diagnosed with VWD, and suggest that mental health screening could be a valuable addition to the care of people diagnosed with VWD.

Abbreviations

CI, confidence interval; CPRD, Clinical Practice Research Datalink; GP, general practitioner; IR, incidence rate; IRR, incidence rate ratio; PY, patient-years; VWD, von Willebrand disease.

Ethical Review and Data Sharing Statement

This study is based on data from the CPRD obtained under license from the UK Medicines and Healthcare products Regulatory Agency. The data are provided by patients and collected by the UK National Health Service (NHS) as part of their care and support. The interpretation and conclusions contained in this study are those of the authors alone. This study was approved by the Independent Scientific Advisory Committee (ISAC) for Medicines and Healthcare products Regulatory Agency database research, and the protocol was made available to the journal reviewers upon request (protocol No: 17_242R2A, Hospital Episode Statistics (HES) Copyright[©] [2018], reused with permission of The Health & Social Care Information Centre. All rights reserved). Researchers can apply for a limited license to access CPRD data for public health research, subject to individual research protocols meeting CPRD data governance

requirements. More details, including data specifications, license fees, and the application process, are available on the CPRD website (<https://www.cprd.com>).

Acknowledgments

Statistical analysis support was provided by Rebecca Persson, MPH, epidemiologist at Boston Collaborative Drug Surveillance Program (Lexington, MA, USA). The authors would like to thank Françoise Truong Berthoz from Takeda Pharmaceuticals International AG, Zurich, Switzerland, and Spiros Tzivelekis from Takeda Development Center Americas, Inc., Cambridge, MA, USA, for their contributions to the study design. Under the direction of the authors, medical writing support was provided by Joanne Vaughan, BSc, employee of Excel Medical Affairs (Fairfield, CT, USA) and was funded by Takeda Development Center Americas, Inc., Lexington, MA, USA.

Data contained within this article were presented in part at the World Federation of Hemophilia Congress; May 8–11, 2022.¹⁹

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was funded by Baxalta US Inc., a Takeda company, Lexington, MA, USA. The sponsor was involved with the study design, interpretation of findings, report development, and in the decision to submit the article for publication.

Disclosure

KWH and SJ have received grant/research support from Baxalta US Inc., a Takeda company, Lexington, MA, USA. GÖ is an employee of Takeda Development Center Americas, Inc., Cambridge, MA, USA, and is a Takeda stockholder. PD was an employee of Takeda Development Center Americas, Inc., Cambridge, MA, USA, at time of study. The authors report no other conflicts of interest in this work.

References

1. Lillicrap D, James P. Von Willebrand disease: an introduction for the primary care physician. Treatment of hemophilia # 47; 2009. Available from: <https://www1.wfh.org/publication/files/pdf-1204.pdf>. Accessed August 8, 2022.
2. Orphanet. Von Willebrand disease; 2020. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=EN&Expert=903. Accessed August 11, 2022.
3. Leebeek FW, Eikenboom JC, Longo DL. Von Willebrand's disease. *N Engl J Med*. 2016;375(21):2067–2080. doi:10.1056/NEJMra1601561
4. Connell NT, Flood VH, Brignardello-Petersen R, et al. ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease. *Blood Adv*. 2021;5(1):301–325. doi:10.1182/bloodadvances.2020003264
5. Nichols WL, Hultin MB, James AH, et al. von Willebrand Disease (VWD): Evidence-based Diagnosis and Management Guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel Report (USA). *Haemophilia*. 2008;14(2):171–232. doi:10.1111/j.1365-2516.2007.01643.x
6. Rae C, Furlong W, Horsman J, et al. Bleeding Disorders, Menorrhagia and Iron Deficiency: Impacts on Health-related Quality of Life. *Haemophilia*. 2013;19(3):385–391. doi:10.1111/hae.12014
7. Xu Y, Deforest M, Grabell J, Hopman W, James P. Relative contributions of bleeding scores and iron status on health-related quality of life in von Willebrand disease: a cross-sectional study. *Haemophilia*. 2017;23(1):115–121. doi:10.1111/hae.13062
8. Roberts JC, Kulkarni R, Kouides PA, et al. Depression and anxiety in persons with von Willebrand disease. *Haemophilia*. 2023;29(2):545–554. doi:10.1111/hae.14725
9. Barlow JH, Stapley J, Ellard DR, Gilchrist M. Information and self-management needs of people living with bleeding disorders: a survey. *Haemophilia*. 2007;13(3):264–270. doi:10.1111/j.1365-2516.2007.01444.x
10. Kirtava A, Drews C, Lally C, Dilley A, Evatt B. Medical, reproductive and psychosocial experiences of women diagnosed with von Willebrand's disease receiving care in haemophilia treatment centres: a case-control study. *Haemophilia*. 2003;9(3):292–297. doi:10.1046/j.1365-2516.2003.00756.x
11. Sidonio RF, Haley KM, Fallaize D. Impact of diagnosis of von Willebrand disease on patient outcomes: analysis of medical insurance claims data. *Haemophilia*. 2017;23(5):743–749. doi:10.1111/hae.13292
12. VanderMeulen H, Arya S, Nersesian S, Philbert N, Sholzberg M. What have we learned about the patient's experience of von Willebrand disease? A focus on women. *Hematology Am Soc Hematol Educ Program*. 2022;2022(1):631–636. doi:10.1182/hematology.2022000391

13. Atiq F, Saes JL, Punt MC, et al. Major differences in clinical presentation, diagnosis and management of men and women with autosomal inherited bleeding disorders. *EClinicalMedicine*. 2021;32:100726. doi:10.1016/j.eclinm.2021.100726
14. Hagberg KW, Jick S, Du P, Truong Berthoz F, Özen G, Tzivelekis S. Impact of von Willebrand disease on women's health outcomes: a matched cohort database study. *J Womens Health*. 2022;31(9):1262–1270. doi:10.1089/jwh.2022.0082
15. Hagberg KW, Divan HA, Nickel JC, Jick SS. Risk of incident antidepressant-treated depression associated with use of 5 α -reductase inhibitors compared with use of α -blockers in men with benign prostatic hyperplasia: a population-based study using the Clinical Practice Research Datalink. *Pharmacotherapy*. 2017;37(5):517–527. doi:10.1002/phar.1925
16. Hagberg KW, Li L, Peng M, Shah K, Paris M, Jick S. Incidence rates of suicidal behaviors and treated depression in patients with and without psoriatic arthritis using the Clinical Practice Research Datalink. *Mod Rheumatol*. 2016;26(5):774–779. doi:10.3109/14397595.2015.1136726
17. Persson R, Lee S, Yood MU, et al. Incident depression in patients diagnosed with multiple sclerosis: a multi-database study. *Eur J Neurol*. 2020;27(8):1556–1560. doi:10.1111/ene.14314
18. Federici AB, Rand JH, Bucciarelli P, et al. Acquired von Willebrand syndrome: data from an international registry. *Thromb Haemost*. 2000;84(2):345–349. doi:10.1055/s-0037-1614018
19. Du P, Hagberg KW, Tzivelekis S, Truong Berthoz F, Özen G, Jick S. Impact of von Willebrand disease on mental health outcomes: a matched cohort database study. *Haemophilia*. 2022;28(Suppl 3):90. doi:10.1111/hae.14537

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Efficacy of rFIXFc versus N9-GP Prophylaxis in Patients with Hemophilia B: Matching-Adjusted Indirect Comparison of B-LONG and PARADIGM 2 Trials

Maria Elisa Mancuso, Daniel Eriksson, Aletta Falk, Zalmai Hakimi, Piotr Wojciechowski, Marlena Wdowiak & Robert Klamroth

To cite this article: Maria Elisa Mancuso, Daniel Eriksson, Aletta Falk, Zalmai Hakimi, Piotr Wojciechowski, Marlena Wdowiak & Robert Klamroth (2023) Efficacy of rFIXFc versus N9-GP Prophylaxis in Patients with Hemophilia B: Matching-Adjusted Indirect Comparison of B-LONG and PARADIGM 2 Trials, Journal of Blood Medicine, , 427-434, DOI: [10.2147/JBM.S389094](https://doi.org/10.2147/JBM.S389094)

To link to this article: <https://doi.org/10.2147/JBM.S389094>



© 2023 Mancuso et al.



Published online: 27 Jul 2023.



Submit your article to this journal



Article views: 243



View related articles



View Crossmark data

Efficacy of rFIXFc versus N9-GP Prophylaxis in Patients with Hemophilia B: Matching-Adjusted Indirect Comparison of B-LONG and PARADIGM 2 Trials

Maria Elisa Mancuso^{1,2}, Daniel Eriksson³, Aletta Falk³, Zalmi Hakimi³, Piotr Wojciechowski^{4,5}, Marlena Wdowiak⁴, Robert Klamroth^{6,7}

¹Center for Thrombosis and Hemorrhagic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy; ²Humanitas University, Pieve Emanuele, Milan, Italy; ³Swedish Orphan Biovitrum AB, Stockholm, Sweden; ⁴Creativ-Ceutical, Krakow, Poland; ⁵Assignity, Krakow, Poland; ⁶Department of Internal Medicine, Hemophilia Treatment Center, Vivantes Klinikum im Friedrichshain, Berlin, Germany; ⁷Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Medical Faculty, University of Bonn, Bonn, Germany

Correspondence: Robert Klamroth, Department of Internal Medicine – Angiology and Hemostaseology, Center for Vascular Medicine, Haemophilia Treatment Center, Vivantes Klinikum im Friedrichshain, Landsberger Allee 49, Berlin, D-10249, Germany, Tel +49 (0)30 130 231575, Fax +49 (0)30 130 232130, Email robert.klamroth@vivantes.de

Purpose: For patients with hemophilia B, extended half-life factor IX (FIX) products are available for prophylaxis and for treating bleeds. Different methods are used to extend the half-lives of recombinant FIX Fc fusion protein (rFIXFc) and nonacog beta pegol (N9-GP). This affects their biodistribution and plasma FIX levels, although differences do not always correlate with clinical outcomes. A matching-adjusted indirect comparison (MAIC) of prophylaxis with rFIXFc and N9-GP was performed, based on licensed dosing in the European Union.

Patients and Methods: Combined rFIXFc data from the weekly and individualized interval prophylaxis arms of the B-LONG clinical trial, and N9-GP data from the 40 IU/kg once-weekly prophylaxis arm of PARADIGM 2 were used in a MAIC. Individual patient data for rFIXFc (n=87) were matched to aggregated data for N9-GP (n=29). Estimated annualized bleeding rates (ABRs) for rFIXFc were recalculated using a Poisson regression model with adjustment for over-dispersion, and compared with ABRs reported for N9-GP, using incidence rate ratios (IRRs) with 95% confidence interval (CI).

Results: There was no evidence of significant differences in estimated ABRs between prophylaxis with rFIXFc and N9-GP. Analysis of pooled rFIXFc weekly and interval-adjusted dosing compared with N9-GP 40 IU/kg once weekly produced estimated ABRs of 2.59 versus 2.51 (IRR 1.03; 95% CI 0.56–1.89), as well as 1.34 versus 1.22 (IRR 1.10; 95% CI 0.42–2.91) and 1.13 versus 1.29 (IRR 0.88; 95% CI 0.47–1.63) for overall, spontaneous, and traumatic bleeding events, respectively.

Conclusion: The study did not reveal any significant differences in the efficacy of rFIXFc and N9-GP prophylaxis. Given differences in trough levels (rFIXFc dosing was targeted to achieve a trough 1–3 IU/dL above baseline versus a reported estimated N9-GP mean trough of 27.3 IU/dL), interpreting plasma FIX levels as potential surrogate efficacy markers requires consideration of compound-specific pharmacokinetic profiles.

Keywords: annualized bleeding rate, factor IX deficiency, factor IX Fc fusion protein, nonacog beta pegol, plasma factor IX activity, treatment outcome

Introduction

For patients with hemophilia B, extended half-life (EHL) factor IX (FIX) products are available for both preventing (prophylaxis) and treating bleeds.¹ These EHL agents display between 3- and 5-fold extension in mean terminal half-life compared with standard FIX products.² Consequently, EHL FIX-based therapies offer the chance of prolonging dosing

intervals between injections, to reduce treatment-related burden while improving bleed protection, thus satisfying most individual patients' needs with favorable short- and long-term outcomes.³ This can benefit patients' quality of life.^{4,5}

Recombinant FIX (rFIX) Fc fusion protein (rFIXFc) is human coagulation FIX covalently linked to the Fc domain of human immunoglobulin G1.⁶ The molecule binds to the neonatal Fc receptor, which is expressed on many cell types throughout life, including endothelial cells lining the vasculature.⁷ The interaction between rFIXFc and the neonatal Fc receptor delays lysosomal degradation, enabling recycling back into the circulation and prolonging the product half-life.⁷ Another therapeutic alternative, nonacog beta pegol (N9-GP), is a human rFIX molecule with a 40 kDa polyethylene glycol (PEG) moiety attached to the activation peptide.⁸ PEGylation reduces the efficiency of various physiologic elimination processes thus favoring the persistence of the molecule in the bloodstream over prolonged time periods.⁹

The different methods used to extend the half-lives of these products affect their biodistribution. Under normal physiologic conditions the majority of FIX is present in the extravascular space.¹⁰ FIX binds to collagen IV in the subendothelial basement membrane, is in dynamic equilibrium with FIX in plasma, and may contribute to hemostasis from its extravascular location.¹⁰ Recent consensus recommendations on management of hemophilia B have highlighted the importance of considering the extravascular distribution of therapeutic products.¹¹ As for unmodified, wild-type FIX,¹² rFIXFc has a high volume of distribution,¹² in line with an extravascular presence, which may be a consequence of the widespread occurrence of cells bearing Fc receptors.¹³ In contrast, likely as a result of conjugation with a PEG moiety,¹⁴ N9-GP has a low volume of distribution.⁸ This indicates that it is mainly limited to the plasma compartment.¹³ Consequently, N9-GP provides higher measurable FIX plasma levels than rFIXFc.¹³ Although FIX plasma levels are commonly used as a surrogate marker of efficacy in hemophilia B, there is considerable variability in inter-patient pharmacokinetic parameters.^{1,15} Levels of plasma FIX activity, which may differ between EHL FIX products, do not always correlate with clinical outcomes and assessment of clinical parameters should be used to help guide clinical decision-making.^{1,13}

This study compares rFIXFc with N9-GP based on the licensed dosing for the two products in the European Union.^{6,8} Both products are approved for treatment and prophylaxis of bleeding in patients with hemophilia B, although use of N9-GP is restricted to patients aged 12 years and above.^{6,8} The recommended starting regimens for prophylaxis with rFIXFc are either 50 IU/kg once weekly (with the dose adjusted based on individual response), or 100 IU/kg once every 10 days (with the interval adjusted based on individual response) for those ≥ 12 years of age.⁶ More frequent or higher doses of rFIXFc may be required in younger individuals.⁶ When N9-GP is used for prophylaxis, 40 IU/kg once weekly is recommended.⁸ Adjustment of dosing and administration intervals of N9-GP may be considered based on FIX levels and individual bleeding tendency.⁸ Different dosing schedules necessarily affect consumption and therefore treatment costs. In addition to clinical outcomes, economic burden is a key aspect of hemophilia B treatment.¹⁶

There are no head-to-head trials directly comparing clinical outcomes in rFIXFc- and N9-GP-treated patients. Also, there are no studies with the same reference arms, precluding indirect comparison through a common comparator. In the absence of these, indirect comparison of trial data using methods for population-adjusted comparison of disconnected intervention, such as simulated treatment comparison¹⁷ or matching-adjusted indirect comparison (MAIC),¹⁸ can be used to assess relative efficacy. In the absence of studies with randomized comparisons against the same treatment or with common comparators, and as disconnected evidence was available, MAIC was identified as the most appropriate technique for this comparison. Using MAIC, the aim of this study was to indirectly compare the efficacy of rFIXFc and N9-GP using efficacy data from two pivotal Phase 3 clinical trials evaluating these products, B-LONG¹² and PARADIGM 2,¹⁹ respectively.

Materials and Methods

Data Sources and Sample Selection

Data for the comparison of rFIXFc with N9-GP were obtained from the two pivotal, multicenter, Phase 3 trials, B-LONG (ClinicalTrials.gov identifier: NCT01027364) and PARADIGM 2 (ClinicalTrials.gov identifier: NCT01333111), respectively, which have been fully reported elsewhere.^{12,19} Both studies involved previously treated male patients (≥ 12 or ≥ 13 years of age in B-LONG and PARADIGM 2, respectively), with factor IX levels ≤ 2 IU/dL.

B-LONG was a non-randomized, open-label study in which 123 patients were enrolled and assigned to one of four rFIXFc treatment groups:¹² weekly prophylaxis (n=63); individualized interval prophylaxis (n=29); on-demand (episodic) treatment (n=27); perioperative management (n=12). Patients assigned to weekly prophylaxis initially received rFIXFc 50 IU/kg, with the dose adjusted as needed. Those assigned to individualized interval prophylaxis initially received rFIXFc 100 IU/kg every 10 days, with the interval adjusted as needed. The dose or interval was adjusted, according to pharmacokinetic data, to maintain a trough level 1–3 IU/dL above baseline, or higher, if clinically necessary.

PARADIGM 2 was a randomized study in which 74 patients received N9-GP either as prophylaxis (fixed dose of 10 IU/kg once weekly [n=30] or 40 IU/kg once weekly [n=29]) or on-demand treatment (n=15).¹⁹ Pharmacokinetic assessments (at initiation and after 12 to 44 weeks of prophylaxis) and the estimation of predose FIX trough levels took place during the study.¹⁹

Based on the licensed dosing regimens for the two products, as described in the respective Summaries of Product Characteristics,^{6,8} the current analysis compares rFIXFc combined data from the weekly and individualized interval prophylaxis arms of B-LONG study with N9-GP data from the 40 IU/kg once-weekly prophylaxis arm of PARADIGM 2.

Outcome Assessment

The efficacy of prophylactic treatment with either rFIXFc or N9-GP was evaluated in terms of estimated annualized bleeding rates (ABRs), considered for any (ie, overall) spontaneous and traumatic bleeds.

Data Analysis

As the trials evaluating rFIXFc and N9-GP have included neither randomized comparisons against the same treatment nor evaluations involving common comparators, neither Bucher's standard methodology for indirect comparison²⁰ nor network meta-analysis^{21,22} were feasible for comparing the two products. Consequently, MAIC was used in accordance with methodologic guidelines for population-adjusted comparison issued by the National Institute for Health and Care Excellence Decision Support Unit (NICE DSU).¹⁷

MAIC is a method of choice for comparing disconnected evidence from different sources.¹⁷ It requires access to patient-level data from at least one of the sources.¹⁷ For this study, (anonymized) individual patient data from B-LONG were available.¹² Aggregated data from the comparator source(s) were collated.^{17,18} In this case, the publicly available patient baseline characteristics and efficacy estimates, quantified in terms of ABR, from PARADIGM 2¹⁹ were used.

When performing MAIC, to adjust the comparison for population differences across trials, the individual patient data are reweighted.^{17,18} Patients with characteristics closer to the aggregated data may be assigned a higher weight, such that the mean characteristics in the reweighted population match the aggregated baseline characteristics of the patients in the comparator trial(s).¹⁷ In this analysis participants receiving rFIXFc prophylaxis were assigned weights to balance between-trial differences for the following characteristics available for PARADIGM 2:¹⁹ age, body weight, the proportions of patients who had prior prophylaxis, white race and Asian ethnicity (which, as the most frequently recorded race/ethnicity classifications in PARADIGM 2, indirectly adjust for others), and the presence of target joints at baseline.

The effects of rFIXFc in the B-LONG study were then recalculated with the use of weights assigned in the previous step, thereby facilitating estimation of the efficacy of rFIXFc had it been administered to the patients in whom the efficacy of N9-GP 40 IU/kg once weekly was determined in PARADIGM 2. In B-LONG, ABRs had originally been determined using negative binomial regression,¹² whereas, in PARADIGM 2 ABRs were estimated using a Poisson regression model, with dose as a factor.¹⁹ To ensure that the method of estimation was fully consistent with PARADIGM 2,¹⁹ ABRs from B-LONG were recalculated using a Poisson regression model accounting for over-dispersed data. The recalculated effects of rFIXFc were compared with the estimated ABRs reported for N9-GP. To facilitate this comparison, incidence rate ratios (IRRs) with 95% confidence interval (CI) were used: logarithmic values of the ABRs for rFIXFc and N9-GP were determined, the difference between these was calculated, and the exponent (power) of this difference was then assessed. If the 95% CI did not include 1.0, statistical significance was considered apparent. Statistical comparisons were performed using R software version 3.5.5 (<https://www.r-project.org/>).

Results

Patient Baseline Characteristics

Ninety-two patients were included in the weekly and individualized interval prophylaxis arms of the B-LONG study. Matching of baseline characteristics with the PARADIGM 2 trial was performed only for those who were included in the B-LONG efficacy analysis (n=87). Of the patients not included in the analysis, five patients were excluded as they did not receive adequate dosing to permit evaluation of rFIXFc prophylaxis, in the weekly prophylaxis arm, one patient did not receive rFIXFc and one received only one dose, and from the individualized interval prophylaxis arm, two received an alternative formulation of rFIXFc and one received only one dose of rFIXFc.¹²

Baseline characteristics of the 87 patients in the rFIXFc prophylaxis arms of the B-LONG study before and after matching with those for the 29 patients assigned to receive N9-GP 40 IU/kg once weekly in PARADIGM 2 are shown in Table 1. Treatment duration was not included in the MAIC evaluation. ABRs were determined over a median of 49.26 weeks in B-LONG and, as defined in the trial design, 52 weeks in PARADIGM 2, time periods may be regarded as a proxy for treatment duration.

At baseline, patients receiving rFIXFc prophylaxis in the B-LONG study had a mean age of 32.8 versus 30.0 years, and a mean body weight of 76.0 versus 70.4 kg compared to individuals assigned prophylaxis with N9-GP 40 IU/kg once weekly in PARADIGM 2. White and Asian patients comprised 62.1% and 16.1%, respectively, of the B-LONG cohort. Corresponding values for PARADIGM 2 were 72.4% and 17.2%, respectively. A numerically lower proportion of the B-LONG (51.7%) than PARADIGM 2 patients had received prior prophylaxis (58.6%), and the proportions with target joints were 49.4% versus 51.7%, respectively.

After adjustment for demographic and disease-related factors, as well as history of treatment, the patient characteristics in both studies were well balanced, and in the current study the effective sample size (ESS) was 59, which corresponded to 68% of the overall population (Figure 1).

Annualized Bleeding Rates

Population-adjusted comparison did not provide evidence of differences in estimated ABRs between prophylaxis with rFIXFc and N9-GP 40 IU/kg once-weekly prophylaxis. Analysis of pooled rFIXFc weekly and interval-adjusted dosing compared with N9-GP 40 IU/kg once weekly produced estimated ABRs of 2.59 versus 2.51 (IRR 1.03; 95% CI 0.56–1.89), as well as 1.34 versus 1.22 (IRR 1.10; 95% CI 0.42–2.91) and 1.13 versus 1.29 (IRR 0.88; 95% CI 0.47–1.63) for any, spontaneous, and traumatic bleeding events, respectively (Figure 2). Additional analyses considering rFIXFc weekly and interval-adjusted prophylaxis separately also showed ABRs to be comparable with N9-GP 40 IU/kg once weekly, with no statistically significant differences shown for IRRs (Supplementary Table 1).

Table 1 Balance of Baseline Characteristics and Effective Sample Size Following Matching of Patients in the B-LONG¹² and PARADIGM 2¹⁹ Phase 3 Studies

	Study Data Prior to Matching		rFIXFc Adjusted Population	
	rFIXFc: B-LONG Baseline (n=87*)	N9-GP: PARADIGM 2 Baseline (n=29 [†])	Estimate	ESS, n (%)
Age in years, mean (SD)	32.82 (14.38)	30.0 (15.8)	30.0 (15.8)	59 (68)
Weight in kg, mean (SD)	75.98 (20.89)	70.4 (15.1)	70.4 (15.1)	
Prior prophylaxis, % of patients	51.7	58.6	58.6	
Race/ethnicity, White, % of patients	62.1	72.4	72.4	
Race/ethnicity, Asian, % of patients	16.1	17.2	17.2	
Target joints, % of patients	49.4	51.7	51.7	

Notes: *Patients included in the efficacy analysis sets for weekly and interval-adjusted rFIXFc prophylaxis; [†]Patients assigned to receive weekly prophylaxis with N9-GP 40 IU/kg.

Abbreviations: ESS, effective sample size; N9-GP, nonacog beta pegol; rFIXFc, recombinant factor IX Fc fusion protein; SD, standard deviation.

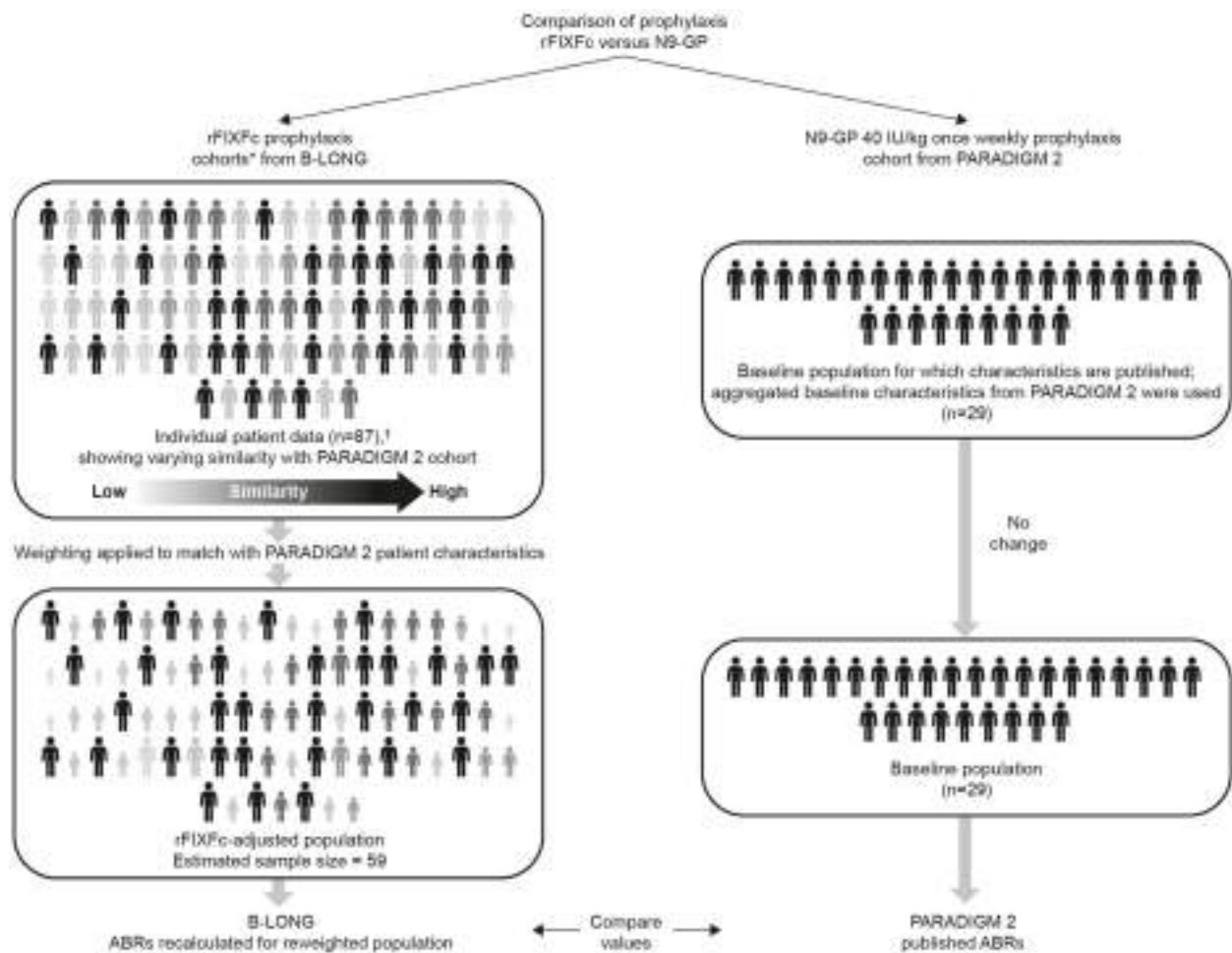


Figure 1 Matching-adjusted indirect comparison of prophylaxis with rFIXFc versus N9-GP, based on data from the B-LONG¹² and PARADIGM 2¹⁹ clinical trials, respectively. *Combined weekly and individualized groups. [†]N=92; five patients were excluded as they did not receive adequate dosing to permit evaluation of rFIXFc prophylaxis.

Abbreviations: ABRs, annualized bleeding rates; N9-GP, nonacog beta pegol; rFIXFc, recombinant factor IX Fc fusion protein.

Discussion

The results of the current indirect treatment comparison, which accounted for baseline differences in the age and weight of the patients in the compared studies, as well as race/ethnicity, any prior prophylaxis, and the presence of target joints, showed no significant difference in efficacy between prophylaxis with rFIXFc and N9-GP, administered as per the licensed dosing.^{6,8} Efficacy, evaluated in terms of the frequency of any, spontaneous, and traumatic bleeding, as measured by estimated by ABRs, was similar for the two products despite differences in trough levels. The target trough levels for rFIXFc prophylaxis were 1–3 IU/dL above baseline,¹² whereas for the N9-GP prophylaxis the observed mean trough activity was estimated at 27.3 IU/dL.¹⁹ As the trough levels for the patients receiving rFIXFc prophylaxis were not a measured endpoint, a more direct comparison of trough levels achieved during the rFIXFc and N9-GP prophylaxis was not possible.

Previously reported product comparison via pharmacokinetic modeling²³ suggests that N9-GP may be well described by a one-compartment model, with the drug remaining mostly in the plasma, while rFIXFc data fit a two- or three-compartment model. This is consistent with the differences in the volumes of distribution between the two products, and in line with rFIXFc exhibiting a similar vascular distribution to wild-type FIX, with an extravascular presence.^{14,23} While the extravascular contribution of rFIXFc remains to be definitively determined, our efficacy results show the value of

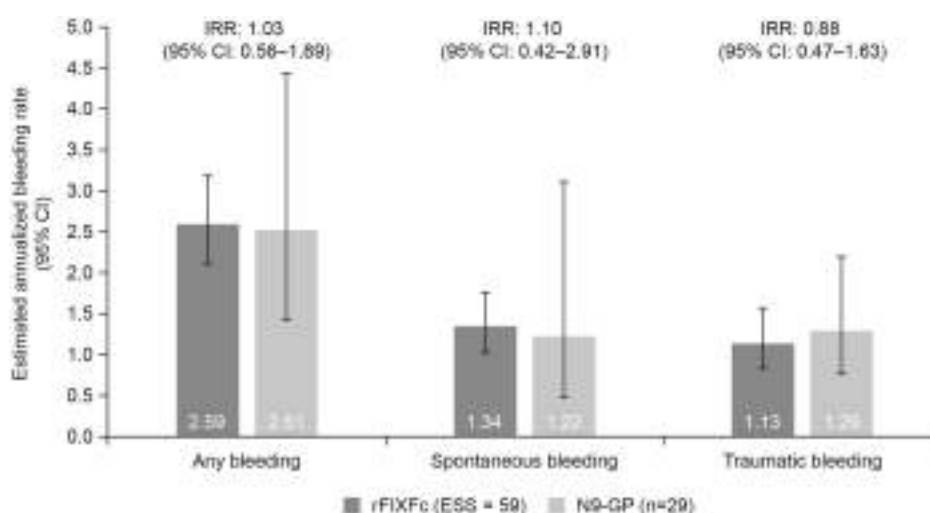


Figure 2 Comparison of estimated annualized bleeding rates for any, spontaneous and traumatic bleeds in patients receiving prophylaxis with rFIXFc versus N9-GP, after matching for baseline variables. Data compare the pooled arms for prophylaxis with rFIXFc (weekly and individualized regimens) in the B-LONG¹² study, adjusted for age, weight, prior prophylaxis, White race, Asian ethnicity and the presence of target joints at baseline, to match those assigned weekly prophylaxis with N9-GP 40 IU/kg in PARADIGM 2.¹⁹

Abbreviations: ESS, effective sample size; CI, confidence interval; IRR, incidence rate ratio; N9-GP, nonacog beta pegol; rFIXFc, recombinant factor IX Fc fusion protein.

using clinical outcomes in addition to plasma FIX activity to help guide clinical decision making. Plasma levels of FIX should be interpreted in the light of the pharmacokinetic characteristics of the therapeutic product used.

With an absence of direct, head-to-head, assessment of clinical outcomes, and as there are no randomized clinical trials in which rFIXFc and N9-GP have been evaluated against the same treatment, to compare the efficacy of these two products, a MAIC¹⁸ was performed. This is an established methodology that has been used for comparing other hemophilia treatments, both for hemophilia A²⁴⁻²⁹ and for hemophilia B,³⁰ but it does have limitations. In the current analysis, the adjusted patient baseline characteristics across the two studies were perfectly matched. However, it is not possible to adjust for all differences, including for unpublished variables of potential relevance, and there is thus the possibility of residual confounding. This is a general feature of such analyses, but with appropriate adjustment the methodology can still be valid. Furthermore, reweighting necessarily decreases the ESS. An adequate sample size is required in the study from which the individual patient data are obtained. In the current study, with a range of key baseline variables reweighted, the ESS for the adjusted B-LONG patient population remained above the sample size of the PARADIGM 2 cohort used in the comparison (59 and 29, respectively). The ESS of 59 was 68% of the initial sample size after matching. This result was an acceptable loss of data with a relatively low risk of bias.¹⁷ Analysis of the patients receiving rFIXFc prophylaxis involved a pooled population of patients receiving both weekly and individualized interval prophylaxis arms. This sample was compared with receipt of N9-GP 40 IU/kg once-weekly prophylaxis. The treatment arms considered in the B-LONG and PARADIGM 2 studies may be regarded as reflective of the recommended dosing strategies for rFIXFc and N9-GP, respectively. Additional analyses considering rFIXFc weekly and interval-adjusted prophylaxis separately also did not reveal any significant differences compared with N9-GP 40 IU/kg once-weekly prophylaxis, although the rFIXFc ESS was necessarily smaller. This may raise some concerns regarding the statistical power of these comparisons. In addition, the current analysis restricted outcome assessment to ABR. It was not possible to compare ABR by previous treatment regimen as the relevant patient characteristics were not reported for PARADIGM 2. Beyond ABR, it would have been valuable to include additional outcomes, such as product consumption, although again, relevant data to facilitate this were not available.

Despite the limitations of the current analysis, this indirect treatment comparison provides useful information about the relative efficacy of prophylaxis with rFIXFc and N9-GP in patients with hemophilia B.

Conclusions

Population-adjusted comparison did not reveal any difference in the efficacy of rFIXFc prophylaxis, weekly/individualized interval, and N9-GP 40 IU/kg once-weekly prophylaxis, regarding the frequency of bleeding events assessed in terms of ABR. Interpretation of plasma FIX levels (1–3 IU/dL targeted with rFIXFc, and an estimated mean of 27.3 IU/dL measured with N9-GP) as potential surrogate markers of efficacy requires consideration of compound-specific pharmacokinetic profiles.

Abbreviations

ABR, annualized bleeding rate; CI, confidence interval; EHL, extended half-life; ESS, estimated sample size; FIX, factor IX; IRR, incidence rate ratio; MAIC, matching-adjusted indirect comparison; N9-GP, nonacog beta pegol; NICE DSU, National Institute for Health and Care Excellence Decision Support Unit; PEG, polyethylene glycol; rFIX, recombinant FIX; rFIXFc, recombinant FIX Fc fusion protein.

Compliance with Ethics Guidelines

Ethical approval was not required for this analysis as it was based on data from two previously published Phase 3 trials, B-LONG and PARADIGM 2. These studies had been performed in line with the Declaration of Helsinki and local regulations, with protocols approved by the authorities and the institutional review board/ethics committee at each participating site. In the original trials, participating patients, or their guardians, provided written informed consent. Further informed consent was not required as the current analysis used de-identified individualized patient-level data from B-LONG (with permission from the dataset owner) and anonymized, previously published data from PARADIGM 2 (with no direct contact with the dataset owner).

Acknowledgments

The project was funded by Sobi. Medical writing and editorial support, funded by Sobi, was provided by Andy Lockley, PhD and Hayley Owen, PhD, Bioscript Medical, Macclesfield, UK. Sobi and Sanofi reviewed and provided feedback on the manuscript.

Author Contributions

All authors made a significant contribution to the reported work: conception; study design and execution; data acquisition, analysis and interpretation; or all of these. The authors all participated in drafting, revising and critically reviewing the article, approving the final version to be published, having agreed on the journal to which the article has been submitted. The authors agree to be accountable for all aspects of the work.

Disclosure

M.E. Mancuso has acted as paid consultant/speaker and/or advisor for Bayer, Biomerin, CSL Behring, Grifols, Kedrion, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sobi, Spark Therapeutics, Takeda and UniQure. D. Eriksson, A. Falk and Z. Hakimi are employees of Sobi. When the analyses described in this manuscript were performed, P. Wojciechowski and M. Wdowiak were both employees of Creativ-Ceutical, a consultancy company that received funding from Sobi for this research. R. Klamroth reports research funding and honoraria for consulting and lectures from Bayer, Biomerin, Biotest, CSL Behring, Chugai, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sobi, Takeda/Shire and UniQure. The authors report no other conflicts of interest in this work.

References

1. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia. *Haemophilia*. 2020;26 Suppl 6:1–158.
2. Lambert T, Benson G, Dolan G, et al. Practical aspects of extended half-life products for the treatment of haemophilia. *Ther Adv Hematol*. 2018;9(9):295–308.
3. Castaman G. The benefits of prophylaxis in patients with hemophilia B. *Expert Rev Hematol*. 2018;11(8):673–683.
4. Astermark J, Hermans C, Ezzalfani M, et al. rFIXFc prophylaxis improves pain and levels of physical activity in haemophilia B: post hoc analysis of B-LONG using haemophilia-specific quality of life questionnaires. *Haemophilia*. 2022;28(1):18–26.

5. Chowdary P, Kearney S, Regnault A, Hoxer CS, Yee DL. Improvement in health-related quality of life in patients with haemophilia B treated with nonacog beta pegol, a new extended half-life recombinant FIX product. *Haemophilia*. 2016;22(4):e267–74.
6. European Medicines Agency. Alprolix® Summary of Product Characteristics. Available from: https://www.ema.europa.eu/en/documents/product-information/alprolix-epar-product-information_en.pdf. Accessed July 18, 2022.
7. Mancuso ME, Mannucci PM. Fc-fusion technology and recombinant FVIII and FIX in the management of the hemophilias. *Drug Des Devel Ther*. 2014;8:365–371.
8. European Medicines Agency. Refixia Summary of Product Characteristics. Available from: https://www.ema.europa.eu/en/documents/product-information/refixia-epar-product-information_en.pdf. Accessed July 18, 2022.
9. Hamidi M, Azadi A, Rafiei P. Pharmacokinetic consequences of pegylation. *Drug Deliv*. 2006;13(6):399–409.
10. Mann DM, Stafford KA, Poon MC, Matino D, Stafford DW. The Function of extravascular coagulation factor IX in haemostasis. *Haemophilia*. 2021;27(3):332–339.
11. Hart DP, Matino D, Astermark J, et al. International consensus recommendations on the management of people with haemophilia B. *Ther Adv Hematol*. 2022;13:20406207221085202.
12. Powell JS, Pasi KJ, Ragni MV, et al. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. *N Engl J Med*. 2013;369(24):2313–2323.
13. Iorio A, Fischer K, Blanchette V, et al. Tailoring treatment of haemophilia B: accounting for the distribution and clearance of standard and extended half-life FIX concentrates. *Thromb Haemost*. 2017;117(6):1023–1030.
14. Preijers T, Bukkems L, van Spengler M, Leebeek F, Nossen M, Mathôt R. In silico comparison of pharmacokinetic properties of three extended half-life factor IX concentrates. *Eur J Clin Pharmacol*. 2021;77(8):1193–1200.
15. Marchesini E, Morfini M, Valentino L. Recent Advances in the Treatment of Hemophilia: a Review. *Biologics*. 2021;15:221–235.
16. Li N, Sawyer EK, Maruszczyk K, et al. Adult lifetime cost of hemophilia B management in the US: payer and societal perspectives from a decision analytic model. *J Med Econ*. 2021;24(1):363–372.
17. Phillippo DM, Ades AE, Dias S, Palmer S, Abrams KR, Welton NJ NICE DSU Technical Support Document 18: methods for population-adjusted indirect comparisons in submission to NICE. Available from: <http://nicedsu.org.uk/wp-content/uploads/2017/05/Population-adjustment-TSD-FINAL.pdf>. Accessed July 18, 2022.
18. Signorovitch JE, Sikirica V, Erder MH, et al. Matching-adjusted indirect comparisons: a new tool for timely comparative effectiveness research. *Value Health*. 2012;15(6):940–947.
19. Collins PW, Young G, Knobe K, et al. Recombinant long-acting glycoPEGylated factor IX in hemophilia B: a multinational randomized phase 3 trial. *Blood*. 2014;124(26):3880–3886.
20. Bucher HC, Guyatt GH, Griffith LE, Walter SD. The results of direct and indirect treatment comparisons in meta-analysis of randomized controlled trials. *J Clin Epidemiol*. 1997;50(6):683–691.
21. Dias S, Sutton AJ, Ades AE, Welton NJ. Evidence synthesis for decision making 2: a generalized linear modeling framework for pairwise and network meta-analysis of randomized controlled trials. *Med Decis Making*. 2013;33(5):607–617.
22. Lumley T. Network meta-analysis for indirect treatment comparisons. *Stat Med*. 2002;21(16):2313–2324.
23. Ecuriola Ettingshausen C, Hegemann I, Simpson ML, et al. Favorable pharmacokinetics in hemophilia B for nonacog beta pegol versus recombinant factor IX-Fc fusion protein: a randomized trial. *Res Pract Thromb Haemost*. 2019;3(2):268–276.
24. Batt K, Gao W, Ayyagari R, et al. Matching-adjusted indirect comparisons of annualized bleeding rate and utilization of BAY 94-9027 versus three recombinant factor VIII agents for prophylaxis in patients with severe hemophilia A. *J Blood Med*. 2019;10:147–159.
25. Bonanad S, Núñez R, Poveda JL, et al. Matching-Adjusted Indirect Comparison of Efficacy and Consumption of rVIII-SingleChain Versus Two Recombinant FVIII Products Used for Prophylactic Treatment of Adults/Adolescents with Severe Haemophilia A. *Adv Ther*. 2021;38(9):4872–4884.
26. Hakimi Z, Santagostino E, Postma MJ, Nazir J. Recombinant FVIIIc Versus BAY 94-9027 for Treatment of Patients with Haemophilia A: comparative Efficacy Using a Matching Adjusted Indirect Comparison. *Adv Ther*. 2021;38(2):1263–1274.
27. Klamroth R, Wojciechowski P, Aballéa S, et al. Efficacy of rFVIIIc versus emicizumab for the treatment of patients with hemophilia A without inhibitors: matching-adjusted indirect comparison of A-LONG and HAVEN Trials. *J Blood Med*. 2021;12:115–122.
28. Pocoski J, Li N, Ayyagari R, et al. Matching-adjusted indirect comparisons of efficacy of BAY 81-8973 vs two recombinant factor VIII for the prophylactic treatment of severe hemophilia A. *J Blood Med*. 2016;7:129–137.
29. Vashi P, Batt K, Klamroth R, et al. Indirect Treatment Comparison of Damocotocog Alfa Pegol versus Turoctocog Alfa Pegol as Prophylactic Treatment in Patients with Hemophilia A. *J Blood Med*. 2021;12:935–943.
30. Astermark J, Wojciechowski P, Aballéa S, Hakimi Z, Nazir J, Klamroth R. Efficacy of rFIXFc versus rIX-FP for the Treatment of Patients with Hemophilia B: matching-Adjusted Indirect Comparison of B-LONG and PROLONG-9FP Trials. *J Blood Med*. 2021;12:613–621.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Systematic Workup of Transfusion Reactions Reveals Passive Co-Reporting of Handling Errors

Elisabeth Nitsche, Jan Dreßler & Reinhard Henschler

To cite this article: Elisabeth Nitsche, Jan Dreßler & Reinhard Henschler (2023) Systematic Workup of Transfusion Reactions Reveals Passive Co-Reporting of Handling Errors, Journal of Blood Medicine, , 435-443, DOI: [10.2147/JBM.S411188](https://doi.org/10.2147/JBM.S411188)

To link to this article: <https://doi.org/10.2147/JBM.S411188>



© 2023 Nitsche et al.



Published online: 08 Aug 2023.



Submit your article to this journal



Article views: 326



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Systematic Workup of Transfusion Reactions Reveals Passive Co-Reporting of Handling Errors

Elisabeth Nitsche^{1,2}, Jan Dreßler^{2,*}, Reinhard Henschler^{1,*}

¹Department of Forensic Medicine, Institute of Legal Medicine, University of Leipzig, Leipzig, Saxony, Germany; ²Department of Quality Control, Institute of Transfusion Medicine, University Hospital Leipzig, Leipzig, Saxony, Germany

*These authors contributed equally to this work

Correspondence: Reinhard Henschler, Institute of Transfusion Medicine, University Hospital Leipzig, Johannisallee 32, Leipzig, Saxony, 04103, Germany, Tel +49 341 97 25300, Email Reinhard.Henschler@medizin.uni-leipzig.de

Introduction: Reporting of transfusion reactions is good practice and required by many guidelines. Errors in the transfusion chain can also lead to severe patient reactions and depend on active error reporting. We aimed to characterize transfusion incidents and asked whether workup of transfusion reactions may also contribute to revealing logistical errors.

Methods: Transfusion medical records from 2011 to 2019 at our tertiary medical centre, as well as forensic autopsy reports, digitized sections, and court records from 1990 to 2019 were analysed. A total of 230,845 components were transfused between 2011 and 2019 at our own institution.

Results: Overall, 322 transfusion incidents were reported. Of these, 279 were from our own institution, corresponding to a frequency of 0.12% of all transfusions. The distribution of reaction types is consistent with the literature, with allergic reactions (55.9%), febrile-non-hemolytic reactions (FNHTR, 24.2%), hemolytic reactions (3.4%) and other types at smaller frequencies (<3%). Twenty-nine (10.4%) of the 279 reports revealed logistical errors, including hemoglobin above guideline threshold (4.3%), incorrect or non-performed bedside tests (3.2%), inadequate patient identification (2.5%), laboratory and issuing errors, missed product checks or failure to follow recommendations (1.1% each). Eight of 29 (27.5%) of the logistical errors were detected by serendipity during workup of incident reports. In addition, 8/932 autopsy cases under code A14 (medical treatment errors) were found to be transfusion-associated (0.9%).

Conclusion: Systematic workup of transfusion incidents can identify previously undetected errors in the transfusion chain. Passive reporting of errors through the recording of side effects may serve as a tool to assess more closely the frequency and quality of handling errors in real life, and thus serve to improve patient safety.

Keywords: adverse transfusion reactions, near miss, hemovigilance, allergic transfusion reaction

Introduction

Blood transfusions rank among the most frequently performed procedures in clinical practice.¹ Despite multiple precautions measures, transfusion-related adverse effects still cause significant morbidity in patients and may also lead to death.^{2–5} Transfusion reactions have been classified into several main types by international consensus criteria such as by the International Haemovigilance Network.^{6–8} The term “transfusion incident” includes, in addition to transfusion reactions, any unintended effect in the patient and/or deviation from established rules and guidelines that occurs before, during or after a transfusion,^{6,9} whereas “near miss” refers to any error that, if not noticed, may lead to faults such as incorrect determination of the blood group or transfusion of the wrong blood product but has been noticed before administration.^{9–11} The recording of handling errors, logistical difficulties and uncertainties as well as near miss events have not been regulated uniformly worldwide and currently lack international standardisation.

The aim of our study was to explore adverse transfusion reactions using a retrospective observational analysis and to identify handling errors and other logistical deficiencies by potential co-reporting in incident reports. This would allow

improving patient safety and provide learning material to further sensitize transfusion staff to the handling of blood products.

Materials and Methods

At our institution, an active error reporting system is in place, with obligatory reporting of all suspected and obvious transfusion incidences. These are discussed with the transfusion officer of the respective department and with the head of the immune hematology laboratory. The reports are finalized and submitted to the supervisory authority by a designated person for pharmaceutical alerts. A total of 322 transfusion incidents, 314 of which had been reported in the Paul Ehrlich Institute documentation form (Langen, Germany, www.pei.de/haemovigilanz-formulare) between January 1, 2011 and December 31, 2019 to the Institute of Transfusion Medicine Leipzig were included in the study. Of these, 279 occurred at the University Hospital Leipzig covering a total of 230,845 transfusions comprising 150,775 erythrocyte concentrates (RCC), 43,247 platelet concentrates (PC) and 36,823 units of frozen fresh plasma (FFP). Another 35 cases were reported by external hospitals and medical practices supplied with blood products from the Institute of Transfusion Medicine Leipzig. Incompletely documented cases (2.8%) were recognized by absence of documented symptoms and excluded from evaluation. Forensic autopsy reports were available for a period from 1990 until 2019 and reported an additional 8 cases related to transfusions in an extended period (in total: 322 cases). Furthermore, all files with the code A14 (medical treatment errors) from 1990 to 2019 at the Institute of Legal Medicine of Leipzig University were consulted ($n = 932$), and a selection of medical records from the years 2010 to 2019 at the Institute of Legal Medicine of the University of Dresden and the Public Prosecutor's Office in the City of Chemnitz were made available by personal communication. Inclusion criteria were documented occurrence or suspicion of a transfusion reaction in the course of the hospital or outpatient unit stay or the presence of a documented transfusion incident evaluated as contributing to the course of the disease. The study protocol was approved by the institutional ethics committee. For statistical analysis, SPSS statistics was used (JMP Statistical Discovery LLC, D- 69188 Heidelberg). Cramér's V calculations were used to measure the effect strength for chi-square tests. Values are between 0 and 1, with higher values indicating a stronger relationship between two nominally scaled variables. Statistical significance was assumed if $p < 0.05$.¹²

Results

Between January 1, 2011 and December 31, 2019, 314 cases of transfusion incidents were reported and filed. A total of 279 cases were reported from the University Hospital, and 35 from other institutions to which blood components were delivered, including other hospitals and external medical practices. At the Institutes of Legal Medicine, 8 autopsy cases were reported as associated with transfusions between 1990 and 2019. Three additional cases in the hospital cohort were associated with death (total, 11). The patients' age ranged from 0 to 92 years ($M = 53.0$ years, median = 60.0, $SD = 24.4$ years). The disease reports originated mainly from hematology (34.4%), from surgical departments (21.5%), or other departments of internal medicine (17.1%; [Figure 1A](#) and [B](#)). The highest proportion of transfusion incidents was reported in standard care wards (78.9%) and intensive/intermediate care wards (13.4%). Other documented locations were outpatient units (3.4%), accident and emergency department (1.6%) and operating theatres (0.9%). In 1.8%, information on the site was missing.

In 80.4% of cases, treating physicians followed the guidelines of the Federal Medical Association¹³ for the workup of transfusion incidents and provided answers to all items. This included laboratory diagnostic parameters, immunohematology and microbiology. In 19.6% of the cases, only partial or no information was provided. As shown in [Table 1](#), indications for transfusions were described most frequently as anemia, thrombocytopenia, and decreased hemoglobin (84.1% of cases). No information on the indication was given in 27 cases (8.4%). Multiple nominations were possible. Clinical symptoms of a reaction after administration of the blood product were documented in 96.3% of incidents. The most common causes were discomfort, increased temperature, chills and urticaria/itching ([Figure 2](#)).

Transfusion reactions were recorded most frequently in the departments of Surgery (19.9%), Internal Medicine (13%) and Intensive Care (11.8%). About 98.4% of all cases were reported as incidents. Three of the incidents were falsely reported as "near miss" events (0.9%), ie they were found to be adverse events, but the report classified them as a near miss. Incidents were noticed by clinical symptoms in 89.4% of the cases. [Table 2](#) shows a breakdown of the types of

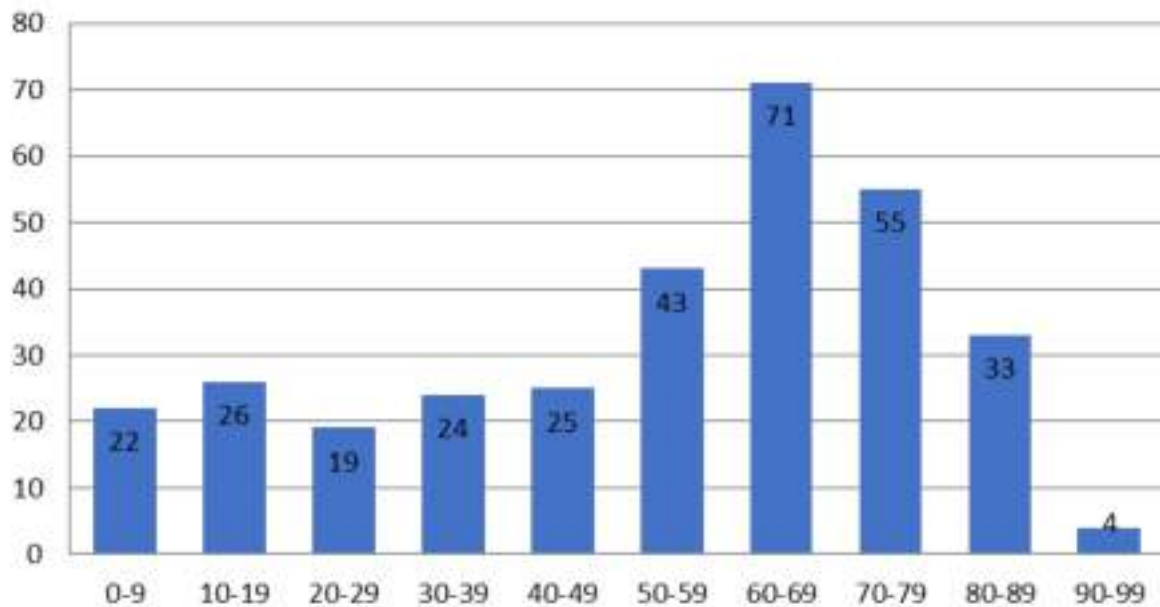
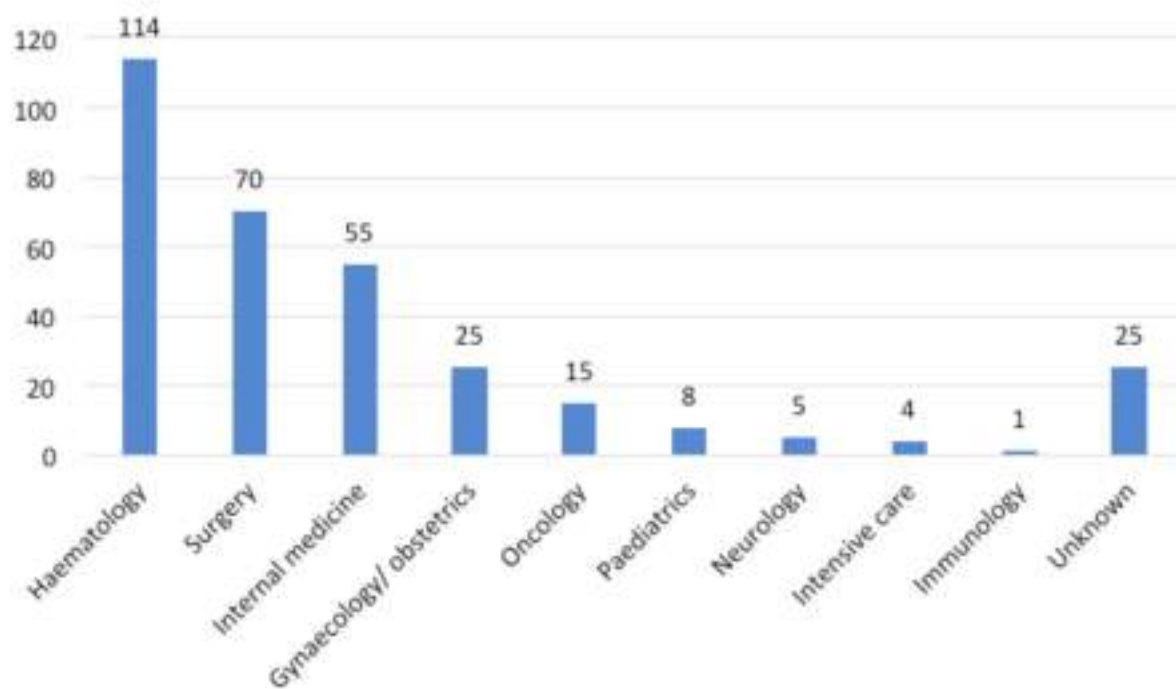
A**B**

Figure 1 Epidemiological parameters and patient distribution. **(A)** Distribution by age of transfusion incidents from 1990 to 2019. **(B)** Distribution of patients by medical discipline. In 25 cases, no information was available.

Table I Distribution of Transfusion Indications in the Study Period

Transfusion Indication	Frequency	Percentage
Anaemia	116	36.0
Thrombocytopenia	98	30.4
Decreasing hemoglobin	57	17.7
Bleeding	27	8.4
Shock	6	1.9
Others	26	8.1
Total	295	91.6
Missing information	27	8.4

reaction. The most commonly classified type of incident was allergic reaction (55.9%), followed by FNHTR (24.2%) and hemolytic reactions (3.4%). Incompletely documented cases resulted in non-classified reactions and occurred in 2.8%. Other types of reactions represented reactions upon suspected causative factors including immunohematological reactions, incompatible cross-matches and bacterial contamination.

Severe reactions were defined when they were either fatal or life-threatening, or accompanied by disability, or when resulting in an extended hospital stay.⁸ Transfusion reactions were classified as non-severe in 49.4% of cases (n = 159) and severe in 24.5% (Table 2). Allergic reactions and FNHTR were the most commonly reported. This was followed by hemolytic events and TACO/TRALI. Information on severity was missing in 26.1% of cases (n = 84). If the patient showed symptoms, but they did not meet the criteria for a transfusion reaction, a transfusion incident was documented as non-causative or unlikely.

Operational errors by staff were found in 29 (10.4%) of the 279 incidents reported from the University Hospital where a total of 230,845 components were transfused, resembling a rate of 1.26 per 10,000 transfusions (0.12%; Table 3). These included transfusion above the upper limit of the hemoglobin threshold (4.3%), omission or incorrect performance of the

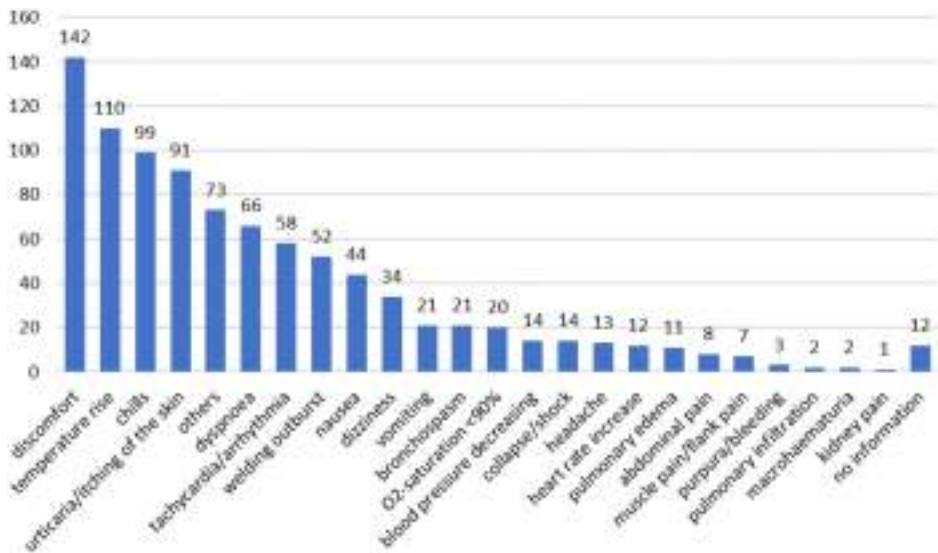


Figure 2 Frequencies of clinical symptoms in patients with reported transfusion incidents. Multiple citations were possible. The category “others” describes individual symptoms of patients that are not classically associated with transfusion events and that are not explicitly listed in the clinical forms and include circulatory instability, joint pain, increased lactate, pallor, edema, cardiac or ophthalmological complaints, cough, hematemesis or neurological symptoms.

Table 2 Distribution of Transfusion Incidents by Type of Reaction

Type of Transfusion Reaction, Including Suspicion Thereof	Number	Percentage	Blood Products Administered			Number of Severe Reactions
			EC	PC	FFP	
Allergic reaction	180	55.9	85	89	11	22
Febrile, non-hemolytic reaction	78	24.2	68	11	0	17
Hemolytic reaction	11	3.4	11	0	0	9
Other types of reaction (immunohematological reactions, unclear interpretation of findings, incompatible cross-matches, bacterial contamination)	11	3.4	6	6	2	6
TACO or TRALI	10	3.1	10	1	1	8
Mistransfusion/ incompatibility	8	2.5	5	1	2	6
Anaphylactic reaction	6	1.9	3	3	0	6
No transfusion reaction/transfusion not causal or unlikely	9	2.8	9	1	0	2
Total	313	97.2	197	112	16	76
Missing information	9	2.8	(n.a.)			3

Abbreviations: EC, Erythrocyte Concentrate; PC, Platelet Concentrate; FFP, Fresh Frozen Plasma; n.a., not applicable.

Table 3 Distribution of Errors and Relative Frequencies

Operational Staff Errors	Number	Frequency (per 10.000 Transfusions)	Percentage of All Reported Incidents
Pretransfusion hemoglobin value above highest guideline threshold	4 (4 [#])	434*	4.3*
Failure or incorrect execution of the bedside test	9 (2 [#])	0.39	3.2
Insufficient identity control of patient and blood product transfused	7 (2 [#])	0.30	2.5
Faulty lab result or wrong product issued	3	0.13	1.1
Product quality not checked	3	0.13	1.1
Transfusion recommendation not followed (warm transfusion with cold agglutinins)	3	0.13	1.1
Total	29 (8[#])	1.26	10.4

Notes: [#]Numbers in brackets indicate accidentally detected errors, ie incidence reports were for medical adverse effects, and only retrospective workup revealed a logistical error; *For the frequency calculation, only RCC transfusions were taken into the calculation, and the value was extrapolated from 4/92 analyzed RCC transfusions. Data were from Transfusion Incident Reports of University Hospital Leipzig.

bedside test (3.2%), insufficient identity control of the blood product and the patient resulting in transfusing the blood product to the wrong patient (2.5%), lab errors, con-compliance with transfusion recommendation (eg warm transfusion) or omitted product quality check (eg expired product; 1.1% each). Operational errors were classified as serious incidents, except for hemoglobin values exceeding the upper guideline limit.

In addition to the cases identified through the hemovigilance system, we analyzed autopsy reports to search for transfusion errors that may have contributed to fatal outcome. Eight cases were identified in the Legal Medicine institutions as associated with a transfusion from a total of 932 section reports between 1990 and 2019. Table 4 shows the diagnoses, symptoms and their potential relation to the transfusion. In one case, transfusion of ABO incompatible RCC was considered a major contributing cause.

Table 4 Legal Medicine Cases Associated with Blood Transfusions and Nature of Reported Transfusion Incidents (Observation Period from 1990 to 2019)

Case	Diagnosis	Type of Blood Product	Type of Reported Transfusion Reaction or Incident	Main Cause of Death
1	Chronic bleeding inflammation of the stomach and duodenum	RCC, PC	Febrile, non-hemolytic reaction	Gastric haemorrhage according to biopsy
2	Benign prostatic hyperplasia	RCC	Wrong blood product to wrong patient	ABO incompatibility, hemolysis, multi-organ failure
3	Chronic pancreatitis	RCC, PC	No classified transfusion reaction/transfusion not causal or unlikely	Dilution coagulopathy
4	Postpartum hemorrhage	RCC, PC, FFP	No classified transfusion reaction	Hemorrhagic shock
5	Osteomyelofibrosis	RCC	TACO/TRALI	Pneumonia
6	Pancreatic cancer	RCC	No classified transfusion reaction	Anemia
7	Femoral fracture	RCC	Wrong blood product to wrong patient	ABO compatible, mismatch
8	Myelodysplastic syndrome	RCC	TACO/TRALI	Anemia

Clinical consequences were derived or recommended in 23.3% of the cases. In 14.7%, the conclusions addressed the organisation of the transfusion chain. These included inspections by the transfusion officer and audits (2.7%), enforcement of close patient monitoring during transfusion (6.7%), references to instructions (8.0%), recommendations for training modules for transfusion staff (5.3%) and case review conferences (2.7%, multiple nominations were possible).

The indication for a red cell transfusion is defined via the pretransfusion hemoglobin value and the patient's hypoxia risk status.^{13,14} In patients without risk factors, a hemoglobin threshold of <7 g/dL (4.3 mmol/L) is considered restrictive. If there are risk factors, it is set between 7 and 10 g/dL (4.3–6.2 mmol/L). The indication for an RCC transfusion according to hemoglobin value <6.2 mmol/L was given in 88/92 (95.7%) of the analysed RCC transfusions studied (Table 5). In 4.3% (4/92 cases with available pretransfusion hemoglobin values), RCC transfusions were not indicated according to the guidelines, representing too high hemoglobin as the most frequent transfusion error.

Statistical analysis explored potential associations between types of component administered, types of reaction, clinical symptoms, severity of symptoms and patients age. Only a moderate effect size (Cramér's $V = 0.427$, $p < 0.001$) was found for the association between RCC administration and the type of transfusion incident, or type of transfusion reaction and administration of FFPs (Cramér's $V = 0.232$, $p = 0.040$) or PCs (Cramér's $V = 0.384$, $p < 0.001$). All other associations were not significant except for the clinical symptoms with FFP (Cramér's $V = 0.176$, $p = 0.002$), incident type and clinical symptoms (Cramér's $V = 0.739$, $p < 0.001$) and severity of the transfusion reaction and RCCs.

Table 5 Available Hemoglobin Values of Patients Before RCC Transfusion

Pretransfusion Hemoglobin		Number of Cases	%
(g/dL)	(mmol/L)		
<7	<4.3	43	51.1
7–9	4.3–6.2	41	46.6
>10	>6.2	4	4.3

Note: All values are in absolute numbers, RCCs were transfused in 92 cases.

Discussion/Conclusions

In this study, we report on a total of 322 transfusion incidents, of which 279 were from our University Hospital representing a total of 230,845 transfusions which allowed frequency calculations. The most frequently reported types of incident were allergic reactions and FNHTR.

Clinical symptoms were reported in 96.3% of the patients and were mostly discomfort (44.1%) or an increase in temperature (34.2%). No symptoms were reported in 3.1% of cases, and in 0.6% documentation was missing. The comparative study by Hatayama et al 2018² reported that clinical symptoms were present in 99.9% of the cases. Here, the largest group was allergic symptoms (urticaria, pruritus, rash) with 68.1%. These figures indicate that the recognition of transfusion incidents might rely on subjective observation and interpretation of symptoms and relating them to the transfusion.

The distribution of the types of adverse transfusion reactions corresponds to internationally reported data, including Serious Hazards of Transfusion (SHOT) reports from the United Kingdom and reports from the International Haemovigilance Network (IHN).^{8,10} For example, allergic reactions and FNHTR were reported in a total of 80.1% of all incidents in our study. Similarly, Saha et al 2020,¹⁵ Goel et al 2019¹⁶ and Moncharmont et al 2018¹⁷ also identified these two types of incident as the largest groups. The observed frequency of incidents of 0.12% in our study is at the lower end of the frequencies described by other authors, which ranked from 0.13% to 2.6%.^{2,15,17–19}

In addition to adverse events, we found actively reported administrative and logistic errors in about 13% of the reports, corresponding to a rate of 1.26/10,000 transfusions (0.12%). Vossoughi et al¹⁸ report an error rate of about 0.15% of all transfusions by active reporting. Similarly, the British SHOT system in the 2021 report²⁰ summarized 2569 errors, which corresponds to a reported error rate of approximately 0.1%. However, SHOT reported a total of 15 cases of ABO incompatible RCC transfusions during the 2018–2021 period, corresponding to about 0.0001% (one per million), whereas our study based on a “passive” co-reporting in cases of transfusion adverse events found three ABO incompatible RCC transfusions in 230,845 transfusions, corresponding to 0.001% (ten per million). Although the numbers are low, this suggests a trend towards a higher rate of ABO incompatible transfusions compared with current SHOT data, and it corresponds to the rate of ABO incompatible RCC transfusions that were measured in the UK SHOT systems in the late 1990s and early 2000s.²⁰

The results of the workup of transfusion adverse event files yielded 8 accidentally detected errors within the 279 reports (2.7%; see Table 3) or of 0.003% (1:28,800) per 230,845 transfusions. This indicates that active reporting of near miss events can be further developed in our hospital, in order to improve patient safety.

Interestingly, we found that staff errors were involved in 29/279 (10.4%) of the reported incidents. Of these, 8 (about one-third; 2.9% of all reported incidents from the University Hospital) were not the cause of the report but were instead detected by serendipity during the workup of the reported incidents. If this rate is applied to all transfusions, this would amount to 6619 errors per 230,845 transfusions. These data point to the substantial potential of systematic workup of transfusion incident reports to detect logistical errors. It demonstrates that the workup of transfusion incidents may reveal information of logistical errors by a “passive” reporting more efficiently than an “active” reporting. Standardized and detailed workup of transfusion reactions can therefore be employed to detect abnormalities and errors and may yield additional detection of incidents reported by an active error reporting.

The second most frequent passively detected error after too high hemoglobin values was the incorrect execution or omission of the bedside test (3.2% of all reported incidents). The bedside test is mandatory in Germany before all RCC transfusions, including emergency transfusions.²¹ This points to deficits in compliance with the written operating procedures and provides a strong basis for additional training of the transfusing personnel.

The limitations of this observational study were that not all reactions were reported in the same way and that some cases were incompletely documented. Transfusion medical files were often lacking at least one parameter ($n = 222$) or were completed in handwritten form. This problem did not occur in the sectional files of the forensic institutes, which were always based on a schema with a defined sequence of documentation and which was available digitally. Reports from the UK SHOT cohort²⁰ describe the benefits of a standardized collection of thematic data. Cases are centrally recorded and evaluated, facilitating comparability with other registries.^{8,20,22} As a consequence at our University Hospital, we therefore introduced a digital documentation instead of hand-written records in order to improve the quality of documentation.

As a conclusion for clinicians, the importance of careful documentation of peri-transfusional data such as hemoglobin value, bedside test and other irregularities should be highlighted in transfusion educational sessions, in the transfusion committee, in audits and in case workups.

The reported transfusion incidents contained 4 actively reported “near miss” events, ie errors noticed before blood products were administered and 8 more detected by serendipity. Here, regulated and efficiency-controlled staff training using teaching examples can raise awareness that valuable clinical conclusions can be drawn from near misses to improve patient safety.^{10,22}

In summary, the results of the present study show the importance of implementing systematic and standardized data acquisition in the context of hemovigilance systems to further expand the understanding of transfusion reactions and thus increase the safety of patients during hemotherapy. Further studies could shed light on the advantages of this approach in an international comparison or collect data beyond the nominal level in order to make even more differentiated statements about patient groups and the relationship between transfusion and transfusion response.

Data Sharing Statement

The data that support the findings of this study are not publicly available because they contain information that could compromise the privacy of research participants but are available from the corresponding author (R.H.).

Statement of Ethics

The research was reviewed for compliance with the guidelines for human studies in accordance with the Declaration of Helsinki of the World Medical Association. A positive ethical vote was obtained from the Ethics Committee of the Medical Faculty of the University of Leipzig (reference: 499/20-ek). Written informed consent was not required, but anonymity was granted.

Author Contributions

E.N. developed the concept of the study, carried out the data collection, analysed the results, wrote the manuscript and created the figures and tables. She agreed on the journal to which the article was submitted. R.H. developed the concept of the study, helped with the acquisition and interpretation of the data, corrected and reviewed the manuscript critically. He agreed on the journal to which the article was submitted. J.D. initiated the study, developed the study design, helped with the acquisition of data and critically reviewed the manuscript. He agreed on the journal to which the article was submitted. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Supported by the Open Access Publishing Fund of Leipzig University.

Disclosure

The authors have no conflicts of interest to declare for this work.

References

1. Murphy MF, Stanworth SJ, Yazer M. Transfusion practice and safety: current status and possibilities for improvement. *Vox Sang*. 2011;100:46–59. doi:10.1111/j.1423-0410.2010.01366.x
2. Hatayama Y, Matsumoto S, Hamada E, et al. Analysis of acute transfusion reactions and their occurrence times. *Yonago Acta Med*. 2018;61:087–090. doi:10.33160/yam.2018.03.013
3. Delaney M, Wendel S, Bercovitz RS, et al. Transfusion reactions: prevention, diagnosis, and treatment. *Lancet*. 2016;388:2825–2836. doi:10.1016/S0140-6736(15)01313-6
4. Pfuntner A, Wier LM, Stocks C. Most frequent conditions in U.S. hospitals, 2011: statistical brief #162; 2021. Available from: <https://europecpmc.org/article/NBK/nbk169248#free-full-text>. Accessed July 13, 2023.

5. Aubron C, Aries P, Le niger C, et al. How clinicians can minimize transfusion-related adverse events? *Transfus Clin Biol*. 2018;25:257–261. doi:10.1016/j.traci.2018.08.158
6. de Vries RRP, Faber JC, Strengers PFW, et al. Haemovigilance: an effective tool for improving transfusion practice. *Vox Sang*. 2011;100:60–67. doi:10.1111/j.1423-0410.2010.01442.x
7. Land KJ, Whitaker BI, Uhl L. National hemovigilance: the current state. In: Cohn CS, Delaney M, Johnson ST, Katz L, editors. *Technical Manual*. 20th ed. Bethesda, MD: AABB; 2023:95–125.
8. Politis C, Wiersum JC, Richardson C, et al. The international haemovigilance network database for the surveillance of adverse reactions and events in donors and recipients of blood components: technical issues and results. *Vox Sang*. 2016;111:409–417. doi:10.1111/vox.12447
9. Nuttall GA, Stubbs JR, We O. Transfusion errors: causes, incidence, and strategies for prevention. *Curr Opin Anaesthesiol*. 2014;27:657–659. doi:10.1097/aco.0000000000000136
10. Narayan S, Poles D; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2019 annual SHOT report (2020); 2021. Available from: <https://www.shotuk.org/wp-content/uploads/myimages/SHOT-REPORT-2019-Final-Bookmarked-v2.pdf>. Accessed July 13, 2023.
11. Maskens C, Downie H, Wendt A, et al. Hospital- based transfusion error tracking from 2005 to 2010: identifying the key errors threatening patient transfusion safety. *Transfusion*. 2014;54:66–73. doi:10.1111/trf.12240
12. Cohen J. Statistical power analysis for the behavioral sciences, New York/ San Francisco/ London, Academic Press (1977); 2021. Available from: https://books.google.de/books?hl=de&lr=&id=rEe0BQAAQBAJ&oi=fnd&pg=PP1&ots=swWRMsURo5&sig=eZonSWt_19_XCEke7o60vbGMzo#v=onepage&q&f=false. Accessed July 13, 2023.
13. Bundesärztekammer. Querschnitts-Leitlinien (BÄK) zur Therapie mit Blutkomponenten und Plasmaderivaten – gesamtnovelle 2020; 2022. Available from: https://www.bundesaeztekammer.de/fileadmin/user_upload/_old-files/downloads/pdf-Ordner/MuE/Querschnitts-Leitlinien_BAEK_zur_Therapie_mit_Blutkomponenten_und_Plasmaderivaten-Gesamtnovelle_2020.pdf. Accessed July 13, 2023.
14. Kotzerke D, Walter Costa M, Voigt J, et al. Novelle QLL 2020 – welche Auswirkungen haben die neu empfohlenen Hämoglobin – transfusionstrigger auf die klinische Versorgung? *Transfusionsmedizin*. 2022;12(01):26–36. doi:10.1055/a-1669-3918
15. Saha S, Krishna D, Prasath R, et al. Incidence and analysis of 7 years adverse transfusion reaction: a retrospective analysis. *Indian J Hematol Blo*. 2020;36:149–155. doi:10.1007/s12288-019-01174-x
16. Goel R, Tobian AA, Shaz BH. Noninfectious transfusion-associated adverse events and their mitigation strategies. *Blood*. 2019;133:1831–1839. doi:10.1182/blood-2018-10-833988
17. Moncharmont P, Barday G, Odent- Malaure H, et al. Adverse transfusion reactions in recipients transfused in out-of-hospital. *Transfus Clin Biol*. 2018;25:105–108. doi:10.1016/j.traci.2018.02.003
18. Vossoughi S, Perez G, Whitaker BI, et al. Safety incident reports associated with blood transfusions. *Transfusion*. 2019;59:2827–2832. doi:10.1111/trf.15429
19. Sousa Neto AL, Barbosa MH. Analysis of immediate transfusion incidents reported in a regional blood bank. *Rev Bras Hematol Hemoter*. 2011;33:337–341. doi:10.5581/1516-8484.20110095
20. Narayan S, Poles D; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2021 annual SHOT report (2022); 2022. Available from: <https://www.shotuk.org/wp-content/uploads/myimages/SHOT-REPORT-2021-FINAL-bookmarked-V2-September.pdf>. Accessed July 13, 2023.
21. Bundesärztekammer. Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (2017); 2021. Available from: https://www.bundesaeztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliH_Lese.pdf. Accessed July 13, 2023.
22. Wood EM, Ang AL, Bisht A, et al. International haemovigilance: what have we learned and what do we need to do next? *Transfus Med*. 2019;29:221–230. doi:10.1111/tme.12582

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Prevalence of Gene Rearrangement by Multiplex PCR in De Novo Acute Myeloid Leukemia in Adult Iraqi Patients

Ali Aljabban & Jaffar Alalsaidissa

To cite this article: Ali Aljabban & Jaffar Alalsaidissa (2023) Prevalence of Gene Rearrangement by Multiplex PCR in De Novo Acute Myeloid Leukemia in Adult Iraqi Patients, Journal of Blood Medicine, , 445-453, DOI: [10.2147/JBM.S416825](https://doi.org/10.2147/JBM.S416825)

To link to this article: <https://doi.org/10.2147/JBM.S416825>



© 2023 Aljabban and Alalsaidissa.



Published online: 11 Aug 2023.



Submit your article to this journal



Article views: 297



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Prevalence of Gene Rearrangement by Multiplex PCR in De Novo Acute Myeloid Leukemia in Adult Iraqi Patients

Ali Aljabban, Jaffar Alalsaidissa

Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq

Correspondence: Ali Aljabban, Department of Pathology, College of medicine, University of Baghdad, Baghdad, Iraq, Tel +9647706056226, Email ali_aljabban@HMS.harvard.com

Introduction: Gene rearrangements of acute myeloid leukemia (AML) play a significant role in categorizing patients and provide valuable information about prognosis and treatment choices. However, in Iraq, the prevalence and prognostic significance of gene rearrangements in AML have not been previously examined.

Methods: This study utilized a multiplex reverse transcription real-time PCR (RT-qPCR) system to identify gene rearrangements in a group of 115 adult patients from Iraq who had been diagnosed with De Novo AML. The diagnosis of AML was confirmed through blood film and flow cytometry. The ethical committee of the College of Medicine at the University of Baghdad provided approval for this research study.

Results: In this study, 66.1% of the patients diagnosed with acute myeloid leukemia (AML) exhibited distinct genetic abnormalities. Among these abnormalities, the most frequent was the rearrangement involving the KMT2A gene, observed in 19.9% of the patients. The risk stratification analysis revealed that 40% of the patients were classified as having a favorable risk, 4.3% as intermediate risk, and 25.2% as adverse risk. A subtype of AML known as core-binding factor (CBF) AML was identified in 21.7% of the cases, with 84% of these patients achieving complete remission. The NPM-RARA gene rearrangement, found in 43% of acute promyelocytic leukemia (APL) cases, was associated with a 71% complete remission rate. Among patients with KMT2A rearrangement, which accounted for 19.9% of all AML cases, the MLL-AF10 rearrangement was the most common, although only one patient with KMT2A rearrangement achieved complete remission. Furthermore, the analysis of demographic data revealed a significant association between increased risk and advanced age, presence of comorbidities, and FAB classification (M0 subtype).

Conclusion: The prevalence of genetic rearrangements in Iraqi De Novo AML patients is higher than the global trend, highlighting the importance of genetic characterization in risk assessment and treatment decisions.

Keywords: gene rearrangement, acute myeloid leukemia, APL, core-binding factor, KMT2A rearrangement

Introduction

Over the past two decades, the classification of acute myeloid leukemia (AML) has undergone significant revisions, shifting towards a greater reliance on the understanding of its genetic landscape. This revolution encompasses various aspects, including chromosomal abnormalities, recurrent gene mutations, and deregulation of microRNAs,¹ which have provided valuable insights into prognosis and treatment response prediction.² The European Leukemia Network (ELN) and National Comprehensive Cancer Network (NCCN) guidelines now recommend comprehensive genetic testing for all newly diagnosed AML patients.^{3,4} This testing includes the screening of gene rearrangements, as it is considered a fundamental requirement for accurately assessing the genetic profile of patients. However, in Iraq, the prevalence and prognostic value of gene rearrangements have not been previously investigated comprehensively. Therefore, the aim of our study is to evaluate the occurrence of gene rearrangements in De Novo AML patients in Iraq and examine their correlation with risk stratification and clinicopathological factors.

Materials and Methods

A prospective cohort study was conducted at Baghdad teaching hospital, involving the recruitment of 115 adult patients who were diagnosed with De Novo AML. The diagnosis of AML was confirmed using blood film and flow cytometry. Data collection was carried out between December 2020 and May 2022. The study was approved by the ethical committee of the College of Medicine, University of Baghdad, and informed consent was obtained from all participating patients.

The inclusion criteria for the study were as follows:

- Patients diagnosed with AML (confirmed by blood film and flow cytometry) within one week after treatment initiation, including newly diagnosed cases.
- Patients aged 18 years and above.

The following were the exclusion criteria:

- Patients who underwent Allogeneic Transplantation.
- Pregnant women.
- Patients with a history of cancer (CA) or myelodysplastic syndrome (MDS).

Method

Peripheral blood samples (3 mL) were obtained from eligible patients using EDTA tubes to ensure sample freshness. RNA extraction was performed using the PAXgene Blood RNA System, which consists of a blood collection tube (PAXgene Blood RNA Tube, BRT) and a nucleic acid purification kit (PAXgene Blood RNA Kit). A comprehensive RT-qPCR-based assay (Leukemia Q-Fusion Screening Kit, Zeesan Biotech Co., Ltd) was utilized to detect more than 140 breakpoints for 30 fusion genes, including *MLL-AF9*, *PML-RARA*, *AML-ETO*, *MLL-AF4*, *TEL-AML1*, *E2A-PBX1*, *MLL-ENL*, *BCR-ABL1*, *SIL-TAL1*, *MLL-AF10*, *CBF-MYH11*, *AML1-MDS1/EV11*, *FIP1L1-PDGFR*, *SET-CAN*, *E2A-HLF*, *DEK-CAN*, *MLL-SEPT6*, *TLS-ERG*, *TEL-PDGFRB*, *MLL-ELL*, *MLL-AF17*, *NPM-RARA*, *NPM-MLF1*, *PLZF-RARA*, *MLL-AF1q*, *MLL-AF1P*, *TEL-ABL*, *AML1-MTG16*, *AML1-EAP*, and *MLL-AF6*.

After RNA extraction, the extracted RNA was combined with pre-made cDNA from the kit. The resulting mixture was divided into eight tubes, each containing specific PCR primers and probes designed for the detection of fusion genes. Real-time qPCR was performed using optical filters to capture fluorescence signals (FAM, HEX, ROX, and Cy5). Samples with a threshold cycle (Ct) value above 25 for Cy5 were considered of low quality and were retested. Samples with a Ct value above 30 were considered to exhibit non-specific amplification and were also repeated. Samples displaying signals with a Ct value below 30 (excluding Cy5) were considered positive for the presence of fusion genes. Furthermore, information regarding *NPM1* and *FLT-3* mutations was obtained from the patients' data.

Ethical Consideration

The study received approval from the scientific committee department of Pathology at the College of Medicine, University of Baghdad, to proceed with the research in compliance with Declaration of Helsinki. Informed consent was obtained from all participants prior to their involvement in the study.

Results

The study included patients with a mean age of 45.1 ± 17.5 years, ranging from 18 to 84 years. The majority of the participants were female (59.1%), and only 16.5% reported being smokers. Among the patients, 2.6% had hypertension, while 7.8% had both hypertension and diabetes mellitus (DM). Splenomegaly was observed in 27.8% of the patients, while hepatomegaly was present in 17.4% of the cases (Table 1).

Table 1 Demographic Characteristics of Included Patients (n=115)

Age	
Mean $\pm$ SD	45.1 $\pm$ 17.5 years
Range	18–84 years
$\leq$ 40 years	49 (42.6%)
41–60 years	39 (33.9%)
> 60 years	27 (23.5%)
Gender, Count (%)	
Male	47 (40.9%)
Female	68 (59.1%)
Smoking, Count (%)	
Yes	19 (16.5%)
No	96 (83.5%)
Comorbidities, Count (%)	
Hypertension	3 (2.6%)
Hypertension & DM	9 (7.8%)
Organomegaly, Count (%)	
Splenomegaly	32 (27.8%)
Hepatomegaly	20 (17.4%)

Abbreviations: DM, Diabetes Mellitus; SD, Standard Deviation.

Gene Rearrangement

The gene rearrangement showed that acute myeloid leukemia with defining genetic abnormalities presented in 66.1% of patients. Among these abnormalities, the most frequent was AML with *KMT2A* rearrangement, accounting for 19.9% of cases. Differentiation-based classification of AML demonstrated that acute myelomonocytic leukemia was the most prevalent subtype, observed in 10 patients (8.7%) (Table 2). The *NPM1* was presented in 33 (28.7%) patients, and *FLT-3* was presented in 18 (15.7%) patients.

Applying the European Leukemia Network (ELN) risk stratification for cytogenetics, the distribution of risk categories was as follows: 25 patients with favorable risk, 5 patients with intermediate risk, and 25 patients with adverse risk. Additionally, considering the presence of the *PML-RARA* fusion, which is associated with a good prognosis, 9 patients were included in the favorable prognosis group. Patients classified as acute myeloid leukemia with differentiation were excluded from this analysis, as their abnormal karyotyping was not assessed in this study. Patients with *FLT-3* mutation alone were categorized as adverse risk, while those with *FLT-3* mutation and other cytogenetic abnormalities followed the cytogenetic risk stratification.⁵ Overall, the study revealed that 46 patients (40%) were classified as favorable risk, 5 patients (4.3%) as intermediate risk, and 29 patients (25.2%) as adverse risk (Table 3).

Follow-Up Period and Response

Each patient was monitored for a duration of one month starting from day 0 of receiving standardized chemotherapy. For statistical purposes, cases of complete remission with incomplete hematological recovery (CRi), five cases, were combined with complete remission (CR). Among the study cohort, a total of 71 (61.74%) patients achieved complete remission, indicating a positive response to treatment. On the other hand, 32 patients (27.8%) experienced treatment failure, characterized by persistence of disease (BP). Unfortunately, there were 12 patients (10.4%) who died before completing the first month of treatment. Out of these, 10 patients refused the treatment, while the remaining two patients passed away within one week of diagnosis.

A significant association was observed between treatment response and risk stratification, indicating that a deterioration in treatment response correlated with a higher risk group ($p=0.0001$) (Table 3). The analysis of demographic data revealed several significant associations. Firstly, advanced age was significantly associated with

Table 2 Prevalence of AML Defining Cytogenetics Abnormalities in Study Cohort According to WHO 2022 Classification

WHO Classification			Count	Percentage
Acute myeloid Leukemia with defining genetic abnormalities	Acute promyelocytic Leukemia with <i>PML-RARA</i> fusion	t(15;17)(q24;q21)	6	5.2%
	Acute promyelocytic Leukemia with <i>PLZF-RARA</i> fusion	t(11;17)(q23;q21)	3	2.6%
	Acute promyelocytic Leukemia with <i>NPM-RARA</i> fusion	t(5;17)(q32;q21)	7	6.1%
	Acute myeloid Leukemia with <i>RUNX1-RUNX1T1</i> fusion	t(8;21)(q22;q22)	19	16.5%
	Acute myeloid Leukemia with <i>CBFB-MYH11</i> fusion	t(16;16)(p13;q22)	6	5.2%
	Acute myeloid Leukemia with <i>DEK-NUP214</i> fusion	t(6;9)(p23;q34)	7	6.1%
	Acute myeloid Leukemia with <i>KMT2A</i> rearrangement	t(10;11)(p12;q23)	15	13%
		t(11;17)(q23;p13)	3	2.6%
		t(9;11)(p22;q23)	5	4.3%
	Acute myeloid Leukemia with <i>NPM1</i> mutation		5	4.3%
Acute myeloid Leukemia, defined by differentiation	Acute myeloid Leukemia with minimal differentiation		7	6.1%
	Acute myeloid Leukemia without maturation		9	7.8%
	Acute myeloid Leukemia with maturation		5	4.3%
	Acute myelomonocytic Leukemia		10	8.7%
	Acute monocytic Leukemia		8	7%

Abbreviations: CBFB-MYH11, Core Binding Factor Beta Subunit-Myosin Heavy Chain 11; DEK-NUP214, DEK Proto-Oncogene-Nucleoporin 214; KMT2A, Myeloid/Lymphoid Or Mixed Lineage Leukemia Gene; NPM, Nucleophosmin; NPM-RARA, Nucleophosmin-Retinoic Acid Receptor Alpha; PLZF-RARA, Promyelocytic Leukemia Zinc Finger-Retinoic Acid Receptor Alpha; PML; RARA, Promyelocytic Leukemia-Retinoic Acid Receptor Alpha; RUNX1-RUNX1T1, Runt-related transcription factor 1-Runt-related transcription factor 1, translocated to 1; WHO, World Health Organization,

Table 3 Relationship of Risk Stratification and Response to Treatment (All Studied Group)

Risk Group (n=115)	Response			Total	P value*
	CR	BP	Death		
Favorable	37 (80.4%)	7 (15.2%)	2 (4.3%)	46 (40%)	0.0001
Intermediate	0	3 (60%)	2 (40%)	5 (4.5%)	
Adverse	8 (27.6%)	16 (55.2%)	5 (17.2%)	29 (25.2%)	
Unclassified	26 (74.3%)	6 (17.1%)	3 (8.6%)	35 (30.4%)	
Total	71 (61.7%)	32 (27.8%)	12 (10.4%)	115 (100%)	

Note: *Chi-Square=33.

Abbreviations: BP, Persistence of Disease; CR, Complete Response.

a higher proportion of adverse risk cases. Secondly, the presence of comorbidities was also significantly associated with adverse risk. Additionally, specific AML subtypes, namely AML-M0, AML-M4, and AML-M5, were significantly associated with adverse risk. Lastly, there was a significant association between the presence of splenomegaly and risk stratification (Table 4).

Core-Binding Factor (CBF) Acute Myeloid Leukemia (AML)

Core-binding factor (CBF) AML is a specific subtype of AML characterized by two distinct genetic abnormalities: t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16). These abnormalities lead to the formation of two fusion genes: *RUNX1/*

Table 4 Relationship of Demographic Data with Risk Stratification for All Patients

		Risk Stratification								P value
		Unclassified		Favorable		Intermediate		Adverse		
		N	%	N	%	N	%	N	%	
Age groups	≤ 40	14	28.6%	29	59.2%	2	4.1%	4	8.2%	0.001
	41–60	16	41.0%	11	28.2%	1	2.6%	11	28.2%	
	> 60	5	18.5%	6	22.2%	2	7.4%	14	51.9%	
Gender	Female	20	29.4%	25	36.8%	2	2.9%	21	30.9%	0.33
	Male	15	31.9%	21	44.7%	3	6.4%	8	17.0%	
Smoking	No	31	32.3%	35	36.5%	5	5.2%	25	26.0%	0.3
	Yes	4	21.1%	11	57.9%	0	0.0%	4	21.1%	
Co-Morbidities	HTN	0	0.0%	0	0.0%	0	0.0%	3	100.0%	0.005
	HTN &DM	3	33.3%	0	0.0%	1	11.1%	5	55.6%	
		No	32	31.1%	46	44.7%	4	3.9%	21	
AML subtype	M0	3	25.0%	0	0.0%	0	0.0%	9	75.0%	0.0001
	M1	9	56.3%	2	12.5%	2	12.5%	3	18.8%	
	M2	5	29.4%	11	64.7%	1	5.9%	0	0.0%	
	M3	0	0.0%	21	100.0%	0	0.0%	0	0.0%	
	M4	10	33.3%	9	30.0%	0	0.0%	11	36.7%	
	M5	8	42.1%	3	15.8%	2	10.5%	6	31.6%	
Splenomegaly	NO	29	34.9%	33	39.8%	5	6.0%	16	19.3%	0.04
	Yes	6	18.8%	13	40.6%	0	0.0%	13	40.6%	
Hepatomegaly	No	33	34.7%	34	35.8%	5	5.3%	23	24.2%	0.07
	Yes	2	10.0%	12	60.0%	0	0.0%	6	30.0%	

RUNX1T1 (also known as *AML1/ETO*) and *CBFB/MYH11*. CBF-AML is known to be associated with a favorable treatment response.

In our analysis, CBF-AML was observed in 21.7% of the patients. Among patients with CBF-AML, 84% achieved complete remission, indicating a positive response to treatment. Only 4 patients (16%) experienced treatment failure, as evidenced by persistent disease (BP). Importantly, no deaths were reported among the patients with CBF AML during the study period.

Acute Promyelocytic Leukemia

Among the patients included in the study, 16 individuals (13.9%) were diagnosed with Acute Promyelocytic Leukemia (APL). The diagnosis of APL was based on the presence of *RARA* translocations, with *PML-RARA* representing 37% of APL cases and *NPM-RARA* representing 43% of APL cases (all patients with *NPM-RARA* also had *NPM1* mutation). Additionally, *PLZF-RARA* was observed in 3 cases (out of 16 APL cases).

There were significant differences observed in the treatment response among patients with APL based on their specific gene rearrangements. For patients with *PML-RARA*, all of them achieved complete remission (CR) as a response to treatment and among patients with *NPM-RARA*, a 71% complete remission rate was observed. In contrast, among the three patients with *PLZF-RARA*, two of them unfortunately died and one experienced treatment failure (BP).

Furthermore, there was a significant difference in treatment response, indicating that patients with *PLZF-RARA* had a worsened response compared to individuals with other gene rearrangements.

Acute Myeloid Leukemia with *KMT2A* Rearrangement

Acute myeloid leukemia with *KMT2A* rearrangement represented in 19.9% of patients. Among the various *KMT2A* rearrangements observed, *MLL-AF10* was the most common (Table 2). However, the response to treatment in patients

with *KMT2A* rearrangement was found to be inadequate. Among these patients, 65% experienced treatment failure (BP), 30% died, and only one patient achieved complete remission (CR).

Notably, there was no significant difference observed in the treatment response between patients with different *KMT2A* rearrangements. However, when comparing the response to treatment with other AML cohorts included in the study, *KMT2A* rearrangement was found to be significantly associated with a poorer response ($p=0.0001$), indicating that patients with *KMT2A* rearrangement have a higher likelihood of an unfavorable treatment outcome compared to patients with other AML genetic abnormalities.

Discussion

The analysis of genetic factors and gene rearrangement revealed that 66.1% of patients with Acute Myeloid Leukemia (AML) exhibited specific genetic abnormalities. On a global scale, cytogenetic abnormalities are observed in approximately 50–60% of newly diagnosed AML patients, and these abnormalities are characterized as non-random chromosomal rearrangements. These aberrations can be further categorized into three groups: AML with balanced translocations/inversions, AML with various cytogenetic abnormalities (such as unbalanced translocations, deletions, monosomies, and trisomies), and AML with complex karyotype (which involves the presence of at least three acquired chromosomal aberrations).⁶ Since in a prospective study, we reported the results of only the first category, the percentage of AML patients with genetic abnormalities tends to be high among Iraqi patients.

In the prospective study, the most common genetic transformation observed was the *RUNX1::RUNX1T1* fusion, which was present in 16% of the AML patients. This finding is consistent with other studies that have reported a prevalence of 10–20% for the *RUNX1::RUNX1T1* fusion in AML cases.⁷ However, it is noteworthy that a previous study conducted in Iraq by Al-Saidi et al reported a lower frequency of the *RUNX1::RUNX1T1* fusion, with only 9% of AML patients exhibiting this genetic abnormality.⁸

The difference in the prevalence of the *RUNX1::RUNX1T1* fusion between the current study and the study by Al-Saidi et al⁸ may be attributed to the different detection methods used. In the current study, PCR-based assays were employed for genetic analysis, which generally provide higher sensitivity and accuracy compared to fluorescence in situ hybridization (FISH) techniques. This difference in detection methods is particularly important in cases with low white blood cell (WBC) counts, where FISH may have reduced accuracy.

Also, a balanced translocation involving *11q23/KMT2A* is among the most frequent chromosomal abnormalities in acute myeloid leukemia. In our study, approximately 20% of AML patients were found to have a translocation involving *11q23/KMT2A*. This prevalence aligns with the findings of the study conducted by Issa et al, which also reported a similar frequency of approximately 20% for this translocation.⁹

In our study, which involved a substantial number of AML patients, we specifically examined cases with t(9;11) and other recurrent balanced rearrangements involving *11q23/KMT2A*. Through our analysis, we identified both similarities and differences among AML patients with these specific translocations. Among the translocations, t(10;11) was found to be the most prevalent, followed by t(11;19), with frequencies ranging from 10% to 12%.^{10–12} These findings shed light on the genetic landscape of AML patients with translocations involving *11q23/KMT2A*, providing insights into the prevalence and specific types of translocations observed. This knowledge contributes to a better understanding of the molecular basis of AML and may have implications for risk stratification and targeted therapeutic approaches in these patients.

In our study, we identified different molecular variants associated with Acute Promyelocytic Leukemia (APL). Specifically, we observed the presence of the *PML-RARA* fusion resulting from the t(15;17)(q24;q21) translocation in 6 (5.2%) patients, the *PLZF-RARA* fusion resulting from the t(11;17)(q23;q21) translocation in 3 (2.6%) patients, and the *PLZF-RARA* fusion resulting from the t(5;17)(q32;q21) translocation in 7 (6.1%) patients. These findings align with previous research by Liquori et al, who reported that the *ZBTB16* (formerly *PLZF*)-*RARA* fusion is the most common molecular variant in APL.¹³

Interestingly, our prospective study revealed a higher prevalence of molecular variants compared to the *PML-RARA* fusion gene in APL cases. This finding contrasts with the results reported by Wen et al, who observed a very low percentage of APL patients with molecular variants.¹⁴ These discrepancies in findings may be attributed to differences in

the detection techniques used and the relatively small sample size of APL cases in our current study. These findings underscore the heterogeneity of APL at the molecular level, with different fusion genes contributing to the disease. Understanding these molecular variants is crucial for accurate diagnosis and targeted treatment approaches in APL patients. Further research with larger sample sizes and comprehensive molecular profiling may provide deeper insights into the prevalence and clinical implications of these molecular variants in APL.

The chromosomal abnormality $\text{inv}(16)(\text{p}13;\text{q}22)$ or $\text{t}(16;16)(\text{p}13.1;\text{q}22)$ is found in approximately 5% to 10% of Acute Myeloid Leukemia (AML) cases.¹⁵ This genetic alteration is characterized by peripheral monocytosis and the infiltration of monoblasts in the bone marrow.¹⁵ In our prospective study, we observed that 5% of AML patients exhibited the *CBFB::MYH11* fusion, which is indicative of $\text{inv}(16)(\text{p}13;\text{q}22)$ or $\text{t}(16;16)(\text{p}13.1;\text{q}22)$.

Upon performing the final risk stratification, we observed that 40% of the patients in our study were classified as having a favorable risk, primarily attributed to the presence of genetic abnormalities such as *RUNX1-RUNX1T1* fusion, *CBFB-MYH11* fusion, and *NPM1* mutation. Intermediate risk was identified in 4.3% of the patients, mainly driven by the *MLLT3-KMT2A* fusion. Adverse risk stratification was assigned to 25.2% of the patients, predominantly due to the presence of genetic abnormalities such as *DEK-NUP214* fusion, *KMT2A* rearrangement, and *FLT-3* mutation. These findings align with the study conducted by Herold et al, which also reported similar percentages for the favorable and adverse risk groups.¹⁶ However, in their study, the intermediate risk group accounted for 20% of the patients. The discrepancy in percentages can be attributed to differences in sample size and the comprehensive molecular and genetic testing performed in their study, which included karyotyping analysis for all patients.

In our prospective study, we observed heterogeneous results among the different risk groups. However, there was a statistically significant increase in the number of deceased patients in the adverse risk group. This finding is consistent with previous studies that have also demonstrated a significant worsening of treatment response among patients classified as having adverse risk stratification.^{17,18} These results support the notion that risk stratification plays a crucial role in predicting treatment outcomes and overall prognosis in patients with acute myeloid leukemia. By identifying patients with adverse risk factors, healthcare providers can better tailor treatment approaches and interventions to improve patient outcomes.

The analysis of demographic data in relation to risk stratification revealed several significant associations. Advanced age, presence of comorbidities, and FAB classification (specifically M0 subtype) were found to be significantly associated with worsening of risk ($p < 0.05$).¹⁹ These findings align with the understanding that AML is more prevalent in the elderly population, and prognosis tends to be poorer in older individuals.

Furthermore, a study by Prassek et al demonstrated that the spectrum and significance of driver gene mutations in older AML patients differ from those in younger patients. In the elderly population, various factors such as the presence of other medical conditions, lower functional ability, and preferences of both physicians and patients for less toxic treatment options often lead to the avoidance of intensive induction chemotherapy in favor of less aggressive therapies.^{20,21}

Taken together, these findings highlight the complex interplay between demographic factors, disease characteristics, and treatment decisions in AML, particularly among elderly patients. The presence of comorbidities and advanced age can contribute to increased risk and influence treatment approaches, emphasizing the need for personalized management strategies in this population.

In the prospective analysis, the prevalence of CBF-AML was found to be higher at 21.7% among the study patients, surpassing the global trend of 12–15%.²² Encouragingly, a significant proportion of patients with CBF-AML achieved complete remission, with an observed rate of 84%. Only 4 patients (16%) experienced treatment failure, while no deaths were reported among those with CBF-AML during the study period. Comparing these outcomes with findings from other study cohorts, CBF-AML demonstrated a more favorable response. It is important to note that CBF-AML generally carries a better prognosis compared to other subtypes of AML. However, it is worth highlighting that despite the favorable prognosis, a subset of CBF-AML patients (approximately 30–40%) may still face the risk of relapse, potentially requiring allogeneic hematopoietic cell transplantation.²³ Therefore, ongoing monitoring and appropriate management remain crucial for achieving long-term remission in CBF-AML cases.

Despite the utilization of All-trans retinoic acid (ATRA) as a treatment approach for APL, which has shown promising results in Iraq,²⁴ notable differences in treatment response were observed in this study. Among patients with APL, all individuals with the *PML-RARA* fusion achieved complete remission CR. However, for patients with the

PLZA-RARA fusion, two out of three patients died, and one experienced treatment failure (BP). In contrast, patients with the *NPM-RARA* fusion exhibited a CR rate of 71%. These findings highlight a significant disparity in treatment response, particularly with respect to patients harboring the *PLZA-RARA* fusion, who exhibited a poorer response compared to other rearrangements.

According to the 2017 ELN recommendations, patients diagnosed with t(9;11) are generally classified as intermediate-risk, regardless of their age or the presence of additional gene mutations. On the other hand, individuals with t(v;11)(v;q23) are categorized as adverse-risk.³ In the current study, patients with *KMT2A* rearrangement exhibited a poor response to treatment, with 65% experiencing treatment failure (BP), 30% of patients died, and only one patient achieving complete remission. Notably, there was no significant difference in treatment response observed among patients with *KMT2A* rearrangement. Compared to all other AML cohorts included in this study, *KMT2A* rearrangement was significantly associated with a poorer treatment response. Furthermore, our analysis revealed that this mutation was more commonly found in patients aged 60 years or older. These findings align with another recently published study, which proposed incorporating age into risk classification. Specifically, the presence of t(9;11) in younger patients may be considered intermediate-risk, whereas its presence in elderly individuals (above 60 years) would warrant adverse-risk stratification.²⁵ These observations emphasize the importance of considering both genetic abnormalities and age when determining risk stratification and treatment approaches for patients with *KMT2A* rearrangement in order to optimize outcomes. Further research is needed to better understand the underlying mechanisms contributing to the poor response associated with *KMT2A* rearrangement and to develop targeted therapies for this specific subgroup of AML patients.

Conclusion

In summary, our prospective analysis of AML patients revealed a high prevalence of specific genetic abnormalities, exceeding the global average for such abnormalities in newly diagnosed AML cases. Additionally, we observed a higher occurrence of molecular variants in APL compared to the commonly studied *PML-RARA* fusion gene. The risk stratification based on genetic factors highlighted the significant associations of age, comorbidities, and FAB classification with risk classification. Notably, our study emphasized the importance of considering age when classifying risk, as the impact of certain genetic abnormalities may differ between younger and older patients. Further research with larger sample sizes is warranted to deepen our understanding of the molecular mechanisms and treatment responses in AML, particularly in subtypes such as APL and AML with *KMT2A* rearrangements.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Yu J, Li Y, Zhang D, Wan D, Jiang Z. Clinical implications of recurrent gene mutations in acute myeloid leukemia. *Exp Hematol Oncol*. 2020;9:4. doi:10.1186/s40164-020-00161-7
2. Hesham Moulod S, Ahmed AL-Rubaie H. April mRNA expression: does it predict the response to induction therapy in adult acute myeloid leukemia? *Biochem Cell Arch*. 2022;22:957–961.
3. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. *J Am Soc Hematol*. 2017;129(4):424–447.
4. National Comprehensive Cancer Network. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Acute Myeloid Leukemia Version 2.2022*. Plymouth Meeting (PA): NCCN; 2022.
5. Tao S, Wang C, Chen Y, et al. Prognosis and outcome of patients with acute myeloid leukemia based on FLT3 ITD mutation with or without additional abnormal cytogenetics. *Oncol Lett*. 2019;18(6):6766–6774. doi:10.3892/ol.2019.11051
6. Pourrajab F, Zare-Khormizi MR, Hashemi AS, Hekmatimoghaddam S. Genetic characterization and risk stratification of acute myeloid leukemia. *Cancer Manag Res*. 2020;12:2231. doi:10.2147/CMAR.S242479
7. Gupta M, Mahapatra M, Saxena R. Cytogenetics' impact on the prognosis of acute myeloid leukemia. *J Lab Physicians*. 2019;11(02):133–137. doi:10.4103/JLP.JLP_164_18
8. AL-Saidi DN, Hameed BM, Khaleel KJ. Detection of RUNX1-RUNX1T1 fusion gene in AML patients by FISH technique in Iraq. *J Commun Dis*. 2021;53(4):54–60.
9. Issa GC, Zarka J, Sasaki K, et al. Predictors of outcomes in adults with acute myeloid leukemia and *KMT2A* rearrangements. *Blood Cancer J*. 2021;11(9):1. doi:10.1038/s41408-021-00557-6
10. Cox MC, Panetta P, Lo-Coco F, et al. Chromosomal aberration of the 11q23 locus in acute leukemia and frequency of MLL gene translocation: results in 378 adult patients. *Am J Clin Pathol*. 2004;122(2):298–306. doi:10.1309/RX27R8GJQM330C22

11. Chen Y, Kantarjian H, Pierce S, et al. Prognostic significance of 11q23 aberrations in adult acute myeloid leukemia and the role of allogeneic stem cell transplantation. *Leukemia*. 2013;27(4):836–842. doi:10.1038/leu.2012.319
12. Bhatnagar B, Blachly JS, Kohlschmidt J, et al. Clinical features and gene-and microRNA-expression patterns in adult acute leukemia patients with t (11; 19)(q23; p13. 1) and t (11; 19)(q23; p13. 3). *Leukemia*. 2016;30(7):1586–1589. doi:10.1038/leu.2015.345
13. Liquori A, Ibañez M, Sargas C, Má S, Barragán E, Cervera J. Acute promyelocytic leukemia: a constellation of molecular events around a single PML-RARA fusion gene. *Cancers*. 2020;12(3):624. doi:10.3390/cancers12030624
14. Wen L, Xu Y, Yao L, et al. Clinical and molecular features of acute promyelocytic leukemia with variant retinoid acid receptor fusions. *Haematologica*. 2019;104(5):e195. doi:10.3324/haematol.2018.205369
15. Pulikkan JA, Castilla LH. Preleukemia and leukemia-initiating cell activity in inv (16) acute myeloid leukemia. *Front Oncol*. 2018;8:129. doi:10.3389/fonc.2018.00129
16. Herold T, Rothenberg-Thurley M, Grunwald VV, et al. Validation and refinement of the revised 2017 European LeukemiaNet genetic risk stratification of acute myeloid leukemia. *Leukemia*. 2020;34(12):3161–3172. doi:10.1038/s41375-020-0806-0
17. Acharya UH, Halpern AB, Wu QV, et al. Impact of region of diagnosis, ethnicity, age, and gender on survival in acute myeloid leukemia (AML). *J Drug Assess*. 2018;7:51–53. doi:10.1080/21556660.2018.1492925
18. Tallman MS, Wang ES, Altman JK, et al. Acute myeloid leukemia, Version 3.2019, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2019;17:721–749. doi:10.6004/jncn.2019.0028
19. Prassek VV, Rothenberg-Thurley M, Sauerland MC, et al. Genetics of acute myeloid leukemia in the elderly: mutation spectrum and clinical impact in intensively treated patients aged 75 years or older. *Haematologica*. 2018;103:1853–1861. doi:10.3324/haematol.2018.191536
20. Rao AV. Fitness in the elderly: how to make decisions regarding acute myeloid leukemia induction. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):339–347.
21. AL-Khalissi KA. Experience with treatment of fifty eight Iraqi patients with Acute Myeloid Leukemia. *JFacMedBagdad*. 2014;55(4):290–295. doi:10.32007/jfacmedbagdad.554566
22. Borthakur G, Kantarjian H. Core binding factor acute myelogenous leukemia-2021 treatment algorithm. *Blood Cancer J*. 2021;11(6):1–5. doi:10.1038/s41408-021-00503-6
23. Ustun C, Morgan E, Moodie EE, et al. Core-binding factor acute myeloid leukemia with t (8; 21): risk factors and a novel scoring system (I-CBF it). *Cancer Med*. 2018;7(9):4447–4455. doi:10.1002/cam4.1733
24. Hussein AG, Ali MS, Hassan MS, Mansoor SS, Ameri AM AL, Muhsin RJ. Haematologic parameters in acute promyelocytic leukemia patients treated with ALL trans- retinoic acid. *J Fac Med Bagdad*. 2007;49(1):51–55.
25. Bill M, Mrózek K, Kohlschmidt J, et al. Mutational landscape and clinical outcome of patients with de novo acute myeloid leukemia and rearrangements involving 11q23/KMT2A. *Proceed National Acad Sci*. 2020;117(42):26340–26346. doi:10.1073/pnas.2014732117

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Multiply Relapsed Secondary CNS Non-Germinal Center Diffuse Large B-Cell Lymphoma Successfully Treated with CNS-Centric Therapy

Lyndsey L Fournier, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Ke Li, Madiha Iqbal, Vivek Gupta & Han W Tun

To cite this article: Lyndsey L Fournier, ErinMarie O Kimbrough, Muhamad Alhaj Moustafa, Ke Li, Madiha Iqbal, Vivek Gupta & Han W Tun (2023) Multiply Relapsed Secondary CNS Non-Germinal Center Diffuse Large B-Cell Lymphoma Successfully Treated with CNS-Centric Therapy, Journal of Blood Medicine, , 455-461, DOI: [10.2147/JBM.S405521](https://doi.org/10.2147/JBM.S405521)

To link to this article: <https://doi.org/10.2147/JBM.S405521>



© 2023 Fournier et al.



Published online: 16 Aug 2023.



Submit your article to this journal



Article views: 259



View related articles



View Crossmark data

Multiply Relapsed Secondary CNS Non-Germinal Center Diffuse Large B-Cell Lymphoma Successfully Treated with CNS-Centric Therapy

Lyndsey L Fournier¹ , ErinMarie O Kimbrough¹, Muhamad Alhaj Moustafa¹ , Ke Li², Madiha Iqbal¹, Vivek Gupta³ , Han W Tun¹ 

¹Division of Hematology and Medical Oncology, Mayo Clinic, Jacksonville, FL, USA; ²Department of Pathology, Mayo Clinic, Jacksonville, FL, USA; ³Department of Radiology, Mayo Clinic, Jacksonville, FL, USA

Correspondence: Han W Tun, Division of Hematology and Oncology, Mayo Clinic, 4500 San Pablo Road S, Jacksonville, FL, 32224, USA, Tel +1 904 953 2693, Fax +1 904 953 2315, Email Tun.Han@mayo.edu

Abstract: Secondary central nervous system involvement by systemic diffuse large B-cell lymphoma (DLBCL) carries a very poor prognosis. We present a female patient who had two episodes of intracerebral central nervous system (CNS)-only relapse of systemic non-germinal center diffuse large B-cell lymphoma (NGC-DLBCL). Her treatment at initial diagnosis consisted of induction with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) and intrathecal (IT) - methotrexate (MTX) followed by consolidation with autologous stem cell transplant (ASCT) after high-dose carmustine, etoposide, cytarabine, and melphalan (BEAM) chemotherapy. She had the first CNS-only relapse 1.5 years post-ASCT and received whole brain radiation therapy (WBRT). She developed the second intracerebral CNS-only relapse 2 years post-WBRT. A CNS-centric therapeutic approach with salvage chemoimmunotherapy incorporating rituximab, high-dose methotrexate (HD-MTX), high-dose cytarabine (HiDAC), and ibrutinib was utilized for her second CNS-only relapse. She underwent consolidation with a second ASCT following high-dose carmustine (BCNU) and thiopeta chemotherapy. Given her high risk of CNS recurrence, she was started on maintenance ibrutinib. To date, she has remained in complete remission for 3 years. In our experience, multiply relapsed secondary CNS lymphoma (SCNSL) with this response is very rare. We suggest one CNS-centric therapeutic approach that can potentially salvage patients with SCNSL who have not had prior exposure to adequate CNS-directed therapies but acknowledge that additional research is necessary to validate our findings.

Keywords: SCNSL, relapsed CNS lymphoma, CNS lymphoma, secondary CNS DLBCL

Introduction

Secondary central nervous system diffuse large B-cell lymphoma (SCNS-DLBCL) represents central nervous system (CNS) dissemination and involvement by a systemic DLBCL at the time of initial diagnosis (de novo SCNSL; DN-SCNSL) or relapse (R-SCNSL).¹⁻⁶ R-SCNSL include CNS-only or concomitant CNS-systemic relapse. R-SCNSL develops early in the disease course with a median time to relapse of 5.4 months from diagnosis. Approximately 80% of patients relapse while receiving chemotherapy or within 6 months of completion of therapy.¹

Among the seventy-five patients enrolled in the largest clinical trial of secondary CNS DLBCL (MARIETTA trial), DN-SCNSL represented 43% of cases, while CNS-only R-SCNSL accounted for 20%, and CNS-systemic R-SCNSL 37% of cases.⁵ The neuroanatomical localization of SCNSL included intracerebral (45%), leptomeningeal (11%), intracerebral/leptomeningeal (17%), intracerebral/ocular (13%), intracerebral/leptomeningeal/ocular (8%), ocular (3%), or spinal cord (3%) disease.⁵ Based on Han's criteria for cell of origin, 59% of the evaluable patients had non-germinal center (NGC)-DLBCL.⁵ The neurological manifestations of SCNSL included motor impairment (49%), sensory impairment (33%), cognitive impairment (20%), sensorial impairment (16%), and language impairment (9%).⁵

SCNSL is extremely uncommon, and there is limited data available regarding optimal treatment. Two of the largest studies reported an incidence of 1.05% for DN-SCNSL and 2.2–2.8% for relapse central nervous system lymphoma (R-SCNSL).^{1,7} While others have suggested that CNS relapse of DLBCL occurs in 3% to 5% of patients with a median overall survival (mOS) of less than 7 months.^{6,8–10} In the studies in which the treatment consisted of intensive CNS-directed therapy and consolidation with autologous stem cell transplant (ASCT), the mOS was less than 10 months with a 3-year overall survival (OS) of 22% for the population as a whole and 42% for patients who underwent consolidation with ASCT has been reported.^{2,11,12} The survival outcome for multiply relapsed SCNSL is not known but likely very poor.

We report a case of multiply relapsed intracerebral SCNSL associated with systemic NGC-DLBCL who was successfully salvaged with CNS-centric therapy with a sustained complete remission (CR) for 3 years to date.

Case

A 38-year-old Asian female presented with a 6-week history of progressive abdominal discomfort and distention, fatigue, 10-pound weight loss, and night sweats. Computed tomography (CT) of the abdomen and pelvis showed extensive soft tissue masses throughout the peritoneal cavity and retroperitoneum. Positron emission tomography-computed tomography (PET-CT) demonstrated generalized hypermetabolic bulky nodal masses including an 8.5×6.9 centimeter (cm) peritoneal mass, a 7.5×6.4 cm pelvic mass, and a 6.3×1.7 cm anterior mediastinal mass. CT guided biopsy of an abdominal mass was performed and demonstrated lambda light chain restricted monoclonal B-cells expressing CD19, CD20, CD45, BCL2, and MUM1. The Ki67 proliferation rate was greater than 90%. Fluorescence in situ hybridization (FISH) showed the *MYC* gene region was within normal limits. The peripheral blood lactate dehydrogenase (LDH) was reportedly elevated at an outside facility. Bone marrow biopsy and cerebrospinal fluid (CSF) evaluations were negative. She was diagnosed with stage IV NGC-DLBCL with a high-intermediate risk age-adjusted international prognostic index (IPI) score given stage IV disease and an elevated LDH at diagnosis. She received 6 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with 4 doses of intrathecal (IT) -methotrexate (MTX) with resolution of her symptoms. Restaging evaluation was consistent with complete remission (CR). After completion of induction therapy, there were concerns among the care team members at the time regarding the high risk of rapid relapse based on aggressiveness of the disease on presentation. The decision was made to proceed with ASCT. She underwent consolidation with high-dose carmustine, etoposide, cytarabine, and melphalan (BEAM) followed by ASCT.

Unfortunately, she developed blurred vision and gait imbalance and was found to have an intracerebral CNS-only relapse 1.5 years following ASCT. Magnetic resonance imaging (MRI) brain showed a 1.7 cm mass in the right foramen of Luschka invading the medulla and right cerebellar tonsil. MRI brain was negative for leptomeningeal enhancement and CSF cytology was negative for lymphoma. CT soft tissue neck, chest, abdomen, and pelvis with contrast showed no evidence of systemic disease. She received whole brain radiation therapy (WBRT) for a total of 2340 cGy with a brain stem boost of 2340 cGy local radiation to the tumor. One month following WBRT, a repeat MRI brain showed a CR.

Two years following WBRT, she presented to our institution with word finding difficulty and mental status changes. MRI of the brain revealed intracerebral, multifocal, enhancing mass lesions with one of the larger components centered within the splenium of the corpus callosum, measuring $1.8 \times 3.5 \times 1.6$ cm (Figure 1). A PET-CT demonstrated a hypermetabolic focus in the left parieto-occipital region suggesting recurrent lymphoma. An excisional biopsy of the left parietal tumor showed large lymphoma cells (Figure 2A). The lymphoma cells were positive for CD20, BCL6, BCL2, and MUM1 by immunohistochemistry (IHC) (Figure 2B–E). They were negative for CD10, CD30, and TdT. Epstein-Barr encoding region (EBER) ish was negative. The Ki67 proliferation index was 90% (Figure 2F). The CSF cytology was negative. These findings were consistent with relapsed CNS NGC-DLBCL according to Hans' algorithm.¹³ Unfortunately, additional testing could not be performed due to the small biopsy.

Salvage chemotherapy with rituximab, high-dose methotrexate (HD-MTX), and high-dose cytarabine (HiDAC) was initiated with improvement in her neurologic symptoms. She developed severe dermatitis which was thought to be secondary to HiDAC. She was transitioned to rituximab, HD-MTX, and Ibrutinib for cycles 2–4 of treatment. Ultimately, the rash was due to acyclovir, and she completed cycles 5–6 with a combination of rituximab, HD-MTX and HiDAC. Subsequent MRI demonstrated a near CR (Figure 3A and B).

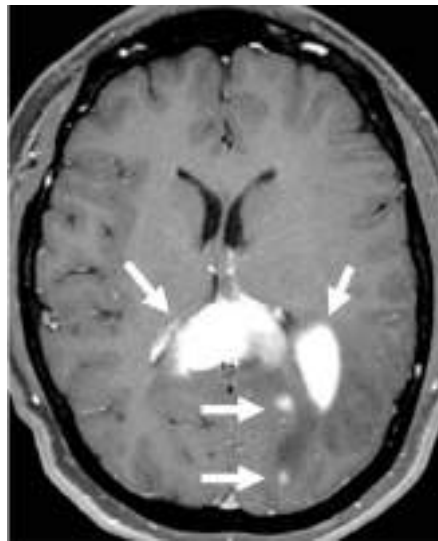


Figure 1 MRI Imaging at Second Relapse. Contrast enhanced axial MRI showing infiltrative, multifocal, enhancing mass lesions with one of the larger components centered within the splenium of the corpus callosum.

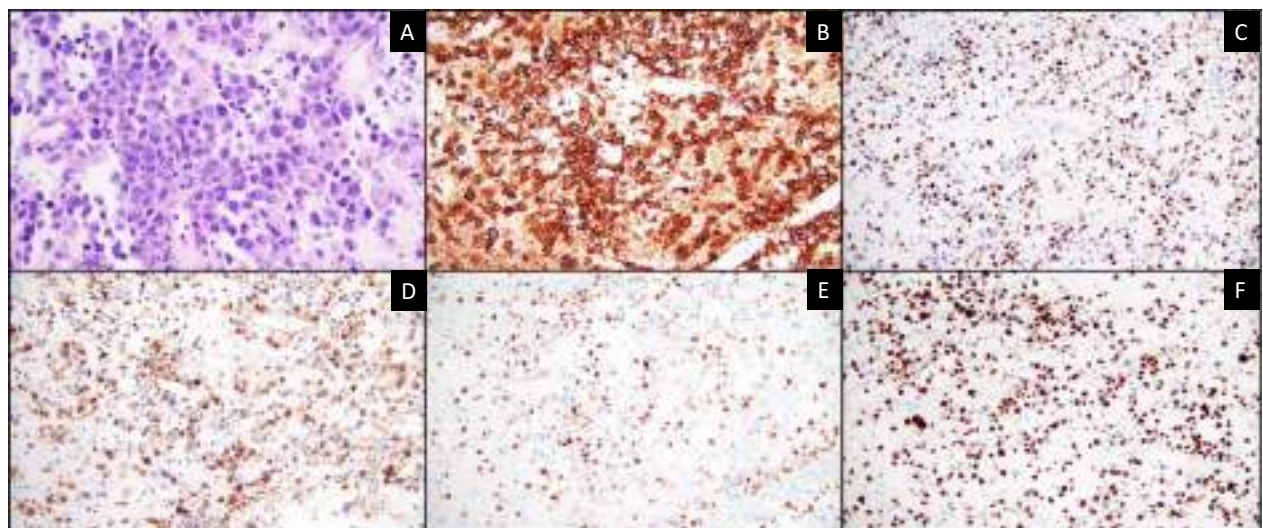


Figure 2 Pathologic Evaluation of the Left Parietal Tumor. H&E x40 magnification shows large B cells (A). IHC x20 magnification shows that the neoplastic B-cells stained positive for CD20 (B), BCL6 (C), BCL2 (D), MUM1 (E) with a high MIB1 proliferation rate (F).

Abbreviations: H&E, haematoxylin and eosin; IHC, immunohistochemistry.

She was consolidated with a second ASCT following high-dose carmustine (BCNU) and thiotepa. Her day 100 work-up including a PET scan, MRI brain and bone marrow biopsy showed a CR (Figure 3C). She was started on maintenance Ibrutinib 560 mg daily due to her high risk of relapse. She has remained in CR for 3 years. She currently enjoys excellent quality of life with full-time employment. A summary of her treatment is provided in Table 1.

Discussion

This case highlights the importance of utilizing CNS-penetrating agents for SCNSL. The patient developed her first CNS-only relapse despite R-CHOP, IT-MTX, and consolidation with ASCT following high dose BEAM chemotherapy. This suggests that the regimen was adequate for her systemic disease but insufficient for the CNS disease. Her second CNS-only relapse occurred after WBRT. This suggests that WBRT alone without systemic CNS-directed therapy is also inadequate. She was successfully

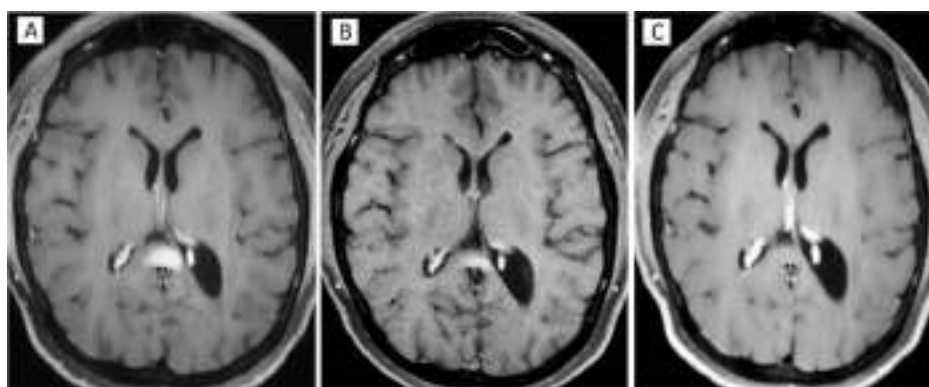


Figure 3 MRI Imaging Post-Treatment. (A and B) MRI after salvage therapy with near complete remission. (C) Repeat contrast enhanced MRI following day 100 work-up status post second ASCT shows complete remission.

Abbreviations: ASCT, autologous stem cell transplant; MRI, magnetic resonance imaging.

salvaged with a combination of CNS-penetrating therapy followed by consolidation with ASCT following CNS-penetrating high dose conditioning chemotherapy. She was then placed on maintenance ibrutinib due to her high risk of CNS relapse. She has been in CR for 3 years to date. The findings in our case indicate that CNS-centric therapy can potentially salvage multiply relapsed SCNSL if the prior systemic treatments do not adequately penetrate the CNS.

Our patient received CNS-directed therapy without systemic disease-directed therapy as she had late-onset CNS-only SCNSL. Most commonly patients with R-SCNSL have early relapse; however, our patient's relapse was more atypical and presented late.¹ Her treatment resembled regimens commonly used for primary CNS lymphoma (PCNSL). In a Phase II clinical trial evaluating patients with SCNSL, induction regimens consisted of CNS-directed as well as systemic disease-directed therapies.^{4,5,14,15} This approach is well suited if the SCNSL has both a CNS and systemic components or if the patient has an early CNS relapse. Therapy directed at systemic disease may not be necessary for CNS-only SCNSL with late relapse. In addition, CNS-directed therapy also impacts systemic disease. Further research is necessary to determine the optimal therapeutic approach for CNS-only SCNSL with late relapse.

Table 1 Case Presentation Treatment Summary

Symptoms	Diagnosis	Induction Regimen	Consolidation Regimen	Maintenance
Presented to outside institution with abdominal pain/distension, fatigue, weight loss, night sweats	Stage IV NGC – DLBCL without BM or CNS involvement	6 cycles R-CHOP 4 doses of IT MTX	Autologous SCT with BEAM conditioning	None
Presented to outside institution with gait imbalance and blurred vision 1.5 years after autologous SCT	First CNS-only relapse of DLBCL	WBRT	None	None
Presented to our hospital with word finding difficulty and mental status changes 2 years post-WBRT	Second CNS- only relapse	Cycle 1: rituximab, HD-MTX, HiDAC. Cycles 2–4: rituximab plus HD-MTX and ibrutinib given rash thought to be due to HiDAC. Rash later determined due to acyclovir. Cycles 5–6: rituximab, HD-MTX, and HiDAC.	Second autologous SCT after conditioning with BCNU plus thiotepa	ibrutinib

Abbreviations: BEAM, carmustine, etoposide, cytarabine, melphalan; BCNU, carmustine; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; HD-MTX, high-dose methotrexate; HiDAC, high-dose cytarabine; IT MTX, intrathecal methotrexate; NGC, non-germinal center; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; SCT, stem cell transplant; WBRT, whole brain radiotherapy.

There have been four phase II clinical trials for SCNSL.^{4,5,14,15} These trials incorporated CNS and systemic disease directed induction followed by consolidation with ASCT/CNS-penetrating high-dose chemotherapy with promising survival outcomes.^{4,5,14,15} The 5-year OS was 41% in the entire study group and 68% in patients who received ASCT.⁴ This strategy was not as effective for R-SCNSL. These patients had a 2-year progression free survival (PFS) of 28% compared to 71% for DN-SCNSL.⁵ Maintenance therapy following ASCT has not been evaluated. A summary of key clinical trials in SCNSL and associated outcomes are included in Table 2.

In regard to WBRT, a retrospective study evaluated response in 25 SCNSL patients who received consolidative versus palliative WBRT. Thirteen patients received consolidative WBRT with a mOS of 24 months and 2-year OS of 64%. The 12 patients who received palliative WBRT had a mOS of 3 months and 2-year OS of 8%. The results of this small study sample suggest that consolidative WBRT may improve long-term survival in SCNSL and could be a potential option for transplant ineligible patients or patients with CNS-only relapse.¹⁶ Additionally, WBRT has been used as the stand-alone treatment in PCNSL with ORR of 90% and median OS of 11.6 months.¹⁷

At the time of first CNS-only relapse, our patient was treated at an outside institution and underwent WBRT. It would have been reasonable to consider treating with CNS-directed systemic therapy instead of WBRT. This is especially true when taking into consideration the neurotoxicity that can result from WBRT including decreased attentiveness and executive function.¹⁸

Our patient has been maintained on continuous ibrutinib therapy due to high risk of relapse. Ibrutinib has been shown to be efficacious in systemic NGC-DLBCL and provides excellent CNS penetration.^{19,20} It has been used successfully in newly diagnosed and relapsed PCNSL.^{21–23} In a Phase I clinical trial, seven patients with R-SCNSL were treated with ibrutinib with an overall response rate of 71%. Approximately 57% of patients experienced a CR, and the median PFS was 7.4 months.²¹ Our patient has been tolerating maintenance ibrutinib quite well.

There is limited data regarding the optimal treatment of multiply relapsed CNS-only SCNSL. This represents just one case and potential treatment strategy. We acknowledge that additional research is necessary to determine the optimal treatment regimen and to assess the role of maintenance therapy.

Table 2 Summary of Clinical Trials in SCNSL and Reported Outcomes

Clinical Trial	Phase	Type of SCNSL	Histology	Treatment	Total Accrual	CR	Survival
Ferreri AJM et al ⁵	2	Initial diagnosis and relapsed	DLBCL	Initial treatment (MATRIX x 3 cycles → RICE x 3 cycles) followed by HD BCNU/TT and ASCT	79	55%	2Y PFS- 46%, 2Y OS- 46%
Ferreri AJM et al ⁴	2	Initial diagnosis and relapsed	DLBCL Blastoid MCL FL (Grade-3)	Initial treatment (R + HDMTX + HiDAC → R-HD sequential chemotherapy with cyclophosphamide, Ara-C, and VP-16) followed by HD BCNU/TT and ASCT	38	63%	2Y EFS- 50%, 5Y OS- 41%
Korfel A et al ¹⁵	2	Relapsed	Aggressive lymphoma (Burkitt lymphoma and lymphoblastic lymphoma excluded)	Initial treatment (MTX/IFO/DEP → Ara-C/TT/DEP) followed by HD BCNU/TT/VP-16 and ASCT	38	63%	2Y TTTF- 49%

Abbreviations: DLBCL, diffuse large b cell lymphoma; MCL, mantle cell lymphoma; FL, follicular lymphoma; MATRIX, High-dose methotrexate + high-dose Ara-C + thiotepa + Rituximab; RICE, Rituximab + ifosfamide + carboplatin + etoposide; BCNU, carmustine; TT, thiotepa; ASCT, autologous stem cell transplant; R, Rituximab; HDMTX, high-dose methotrexate; HiDAC, high-dose ara-c; VP-16, etoposide; MTX, methotrexate; IFO, ifosfamide; DEP, liposomal ara-c; CR, complete response; PFS, progression free survival; OS, overall survival; EFS, event free survival; TTTF, time to treatment failure.

Conclusion

CNS-directed therapy is critical in the management of SCNSL. Our patient achieved a long-term CR after salvage CNS-directed treatment for multiply relapsed intracerebral CNS-only SCNSL. The therapeutic success in our patient is likely due to high-dose BCNU/Thiotepa chemotherapy with ASCT and maintenance ibrutinib. This case demonstrates the benefit of CNS-centric salvage therapy in multiply relapsed SCNSL when the patient has previously received inadequate systemic CNS-directed therapy. For eligible SCNSL patients, induction therapy and high-dose chemotherapy consisting of CNS-penetrating agents should be considered. Current therapies for R-SCNSL are limited and are often ineffective. Novel therapies are urgently needed for elderly and unfit patients who are not eligible for currently available intensive therapies. In addition, the role of maintenance therapy needs to be further explored. Additional research is necessary to assess the therapeutic approach which was undertaken in our patient with multiply relapsed SCNSL.

Abbreviations

ASCT, autologous stem cell transplant; BCNU, carmustine; BEAM, carmustine, etoposide, cytarabine, melphalan; cm, centimeter; CNS, central nervous system; CR, complete remission; CSF, cerebrospinal fluid; CT, computed tomography; DN-SCNSL, de novo secondary CNS lymphoma; DLBCL, diffuse large b-cell lymphoma; EBER, Epstein-Barr encoding region; FISH, fluorescence in situ hybridization; HiDAC, high-dose cytarabine; HD-MTX, high-dose methotrexate; IHC, immunohistochemistry; IPI, international prognostic index; IT, intrathecal; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; MTX, methotrexate; mOS, median overall survival; NGC, non-germinal center; NGC-DLBCL, non-germinal center DLBCL; OS, overall survival; PET-CT, positron emission tomography-computed tomography; PCNSL, primary CNS lymphoma; PFS, progression free survival; R-CNSL, relapse central nervous system lymphoma; R-SCNSL, relapse secondary CNS lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; SCNSL, secondary CNS lymphoma; SCNS-DLBCL, secondary CNS diffuse large B-cell lymphoma; WBRT, whole brain radiation therapy.

Consent for Publication

The study participant has given written informed consent to participate as well as written informed consent to publish the case details and accompanying images. Institutional approval was not required to publish the case details.

Disclosure

The authors report no conflicts of interest in this work.

References

- Bernstein SH, Unger JM, LeBlanc M, et al. Natural history of CNS relapse in patients with aggressive non-Hodgkin's lymphoma: a 20-year follow-up analysis of SWOG 8516 – the southwest oncology group. *J Clin Oncol*. 2009;27(1):114–119. doi:10.1200/JCO.2008.16.8021
- Bromberg JE, Doorduijn JK, Illerhaus G, et al. Central nervous system recurrence of systemic lymphoma in the era of stem cell transplantation—an international primary central nervous system lymphoma study group project. *Haematologica*. 2013;98(5):808–813. doi:10.3324/haematol.2012.070839
- El-Galaly TC, Cheah CY, Bendtsen MD, et al. Treatment strategies, outcomes and prognostic factors in 291 patients with secondary CNS involvement by diffuse large B-cell lymphoma. *Eur J Cancer*. 2018;93:57–68. doi:10.1016/j.ejca.2018.01.073
- Ferreri AJ, Donadoni G, Cabras MG, et al. High doses of antimetabolites followed by high-dose sequential chemoimmunotherapy and autologous stem-cell transplantation in patients with systemic B-cell lymphoma and secondary CNS involvement: final results of a multicenter phase II trial. *J Clin Oncol*. 2015;33(33):3903–3910. doi:10.1200/JCO.2015.61.1236
- Ferreri AJM, Doorduijn JK, Re A, et al. MATRix-RICE therapy and autologous haematopoietic stem-cell transplantation in diffuse large B-cell lymphoma with secondary CNS involvement (MARIETTA): an international, single-arm, Phase 2 trial. *Lancet Haematol*. 2021;8(2):e110–e121. doi:10.1016/S2352-3026(20)30366-5
- Klanova M, Sehn LH, Bence-Bruckler I, et al. Integration of cell of origin into the clinical CNS international prognostic index improves CNS relapse prediction in DLBCL. *Blood*. 2019;133(9):919–926. doi:10.1182/blood-2018-07-862862
- Boehme V, Zeynalova S, Kloess M, et al. Incidence and risk factors of central nervous system recurrence in aggressive lymphoma—a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). *Ann Oncol*. 2007;18(1):149–157. doi:10.1093/annonc/mdl327
- Savage KJ, Slack GW, Mottok A, et al. Impact of dual expression of MYC and BCL2 by immunohistochemistry on the risk of CNS relapse in DLBCL. *Blood*. 2016;127(18):2182–2188. doi:10.1182/blood-2015-10-676700
- Schmitz N, Zeynalova S, Nickelsen M, et al. CNS international prognostic index: a risk model for CNS relapse in patients with diffuse large B-cell lymphoma treated with R-CHOP. *J Clin Oncol*. 2016;34(26):3150–3156. doi:10.1200/JCO.2015.65.6520

10. Sehn LH, Salles G. Diffuse large B-cell lymphoma. *N Engl J Med*. 2021;384(9):842–858. doi:10.1056/NEJMra2027612
11. Kasamon YL, Jones RJ, Piantadosi S, et al. High-dose therapy and blood or marrow transplantation for non-Hodgkin lymphoma with central nervous system involvement. *Biol Blood Marrow Transplant*. 2005;11(2):93–100. doi:10.1016/j.bbmt.2004.09.009
12. Williams CD, Pearce R, Taghipour G, et al. Autologous bone marrow transplantation for patients with non-Hodgkin's lymphoma and CNS involvement: those transplanted with active CNS disease have a poor outcome—a report by the European bone marrow transplant lymphoma registry. *J Clin Oncol*. 1994;12(11):2415–2422. doi:10.1200/JCO.1994.12.11.2415
13. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275–282. doi:10.1182/blood-2003-05-1545
14. Doorduijn JK, van Imhoff GW, van der Holt B, et al. Treatment of secondary central nervous system lymphoma with intrathecal rituximab, high-dose methotrexate, and R-DHAP followed by autologous stem cell transplantation: results of the HOVON 80 phase 2 study. *Hematol Oncol*. 2017;35(4):497–503. doi:10.1002/hon.2342
15. Korfel A, Elter T, Thiel E, et al. Phase II study of central nervous system (CNS)-directed chemotherapy including high-dose chemotherapy with autologous stem cell transplantation for CNS relapse of aggressive lymphomas. *Haematologica*. 2013;98(3):364–370. doi:10.3324/haematol.2012.077917
16. Walburn T, Grover NS, Shen CJ, et al. Consolidative or palliative whole brain radiation for secondary CNS diffuse large B-Cell lymphoma. *Leuk Lymphoma*. 2021;62(1):68–75. doi:10.1080/10428194.2020.1821014
17. Nelson DF, Martz KL, Bonner H, et al. Non-Hodgkin's lymphoma of the brain: can high dose, large volume radiation therapy improve survival? Report on a prospective trial by the Radiation Therapy Oncology Group (RTOG): RTOG 8315. *Int J Radiat Oncol Biol Phys*. 1992;23(1):9–17. doi:10.1016/0360-3016(92)90538-S
18. Ferreri AJM, Cwynarski K, Pulczynski E, et al. Long-term efficacy, safety and neurotolerability of MATRix regimen followed by autologous transplant in primary CNS lymphoma: 7-year results of the IELSG32 randomized trial. *Leukemia*. 2022;36(7):1870–1878. doi:10.1038/s41375-022-01582-5
19. Lionakis MS, Dunleavy K, Roschewski M, et al. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma. *Cancer Cell*. 2017;31(6):833–843 e5. doi:10.1016/j.ccell.2017.04.012
20. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med*. 2015;21(8):922–926. doi:10.1038/nm.3884
21. Grommes C, Pastore A, Palaskas N, et al. Ibrutinib Unmasks Critical Role of Bruton Tyrosine Kinase in Primary CNS Lymphoma. *Cancer Discov*. 2017;7(9):1018–1029. doi:10.1158/2159-8290.CD-17-0613
22. Kuhlman JJ, Alhaj Moustafa M, Jiang L, et al. Long-term survival with ibrutinib therapy in elderly patients with newly diagnosed primary central nervous system lymphoma. *Blood Lymphat Cancer*. 2022;12:23–29. doi:10.2147/BLCTT.S360442
23. Soussain C, Choquet S, Blonski M, et al. Ibrutinib monotherapy for relapse or refractory primary CNS lymphoma and primary vitreoretinal lymphoma: final analysis of the phase II 'proof-of-concept' iLOC study by the Lymphoma study association (LYSA) and the French oculo-cerebral lymphoma (LOC) network. *Eur J Cancer*. 2019;117:121–130. doi:10.1016/j.ejca.2019.05.024

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Hematological Abnormalities Among Malaria Infected Adult Patients in Association with ABO Blood Groups at Jinella Health Center, Harar, Eastern Ethiopia

Haftu Asmerom, Kabtamu Gemechu, Beza Sileshi & Mesay Arkew

To cite this article: Haftu Asmerom, Kabtamu Gemechu, Beza Sileshi & Mesay Arkew (2023) Hematological Abnormalities Among Malaria Infected Adult Patients in Association with ABO Blood Groups at Jinella Health Center, Harar, Eastern Ethiopia, Journal of Blood Medicine, , 463-476, DOI: [10.2147/JBM.S419815](https://doi.org/10.2147/JBM.S419815)

To link to this article: <https://doi.org/10.2147/JBM.S419815>



© 2023 Asmerom et al.



Published online: 21 Aug 2023.



Submit your article to this journal



Article views: 475



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Hematological Abnormalities Among Malaria Infected Adult Patients in Association with ABO Blood Groups at Jinella Health Center, Harar, Eastern Ethiopia

Haftu Asmerom , Kabtamu Gemechu , Beza Sileshi, Mesay Arkew 

School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

Correspondence: Haftu Asmerom, School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, P.O. Box: 235, Harar, Ethiopia, Tel +251910089878, Email wawhaftu12@gmail.com

Background: Hematological abnormalities are a common complication of malaria infection. However, there is a paucity of evidence regarding it among malaria-infected adult patients in association with the ABO blood group in Ethiopia, particularly in the Harari Region. Therefore, this study aimed to assess the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia.

Methods: An institutional-based cross-sectional study was conducted from July 10, 2022, to January 10, 2023. Four milliliters of venous blood were collected from each study participant. Drops of blood were used for blood film preparation. ABO blood group was determined by agglutination test using monoclonal anti-sera (Agape Diagnostics Ltd., India). A complete blood count was done using the DxH 800 (Beckman Coulter, Inc, Miami, FL) hematology analyzer. The data were analyzed using SPSS version 26. Bivariable and multivariable logistic regression models were fitted. The level of significance was declared at a p-value of <0.05.

Results: The study revealed that 47.2% (95% CI: 41.0–53.6) of the participants were anemic. Being female (AOR = 3.18, 95% CI = 1.67, 6.04), having the A blood group (AOR = 2.75, CI = 1.20, 6.31), and being infected with *P. falciparum* (AOR = 2.64, CI = 1.26, 5.53) were all significantly associated with malaria anemia. The overall prevalence of thrombocytopenia was also 67.7% (95% CI: 61.7–73.4%). It was significantly associated with *P. falciparum* infection (AOR = 8.03, CI = 3.53, 18.25) and high parasitemia levels (AOR = 4.40, CI = 1.57, 12.32).

Conclusion: Patients with malaria who belonged to the “A” blood group in the study area had anemia as a serious health problem. Hence, frequently checking for anemia in patients with malaria who have blood group “A” can help with early detection and better management of anemia.

Keywords: malaria, hematological abnormalities, blood groups, Harar, Ethiopia

Background

The two *Plasmodium* species, *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) are the principal causes of malaria and pose the greatest risks to the world’s population.¹ The most frequent side effects of malaria infection are hematological abnormalities, which affect red blood cells (RBC), white blood cells (WBC), and platelets (PLT).^{2,3}

Hematological abnormalities are caused by *P. falciparum*-encoded polypeptides (*RIFINs*), sub-telomeric variants (*STEVAR*), and *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*) to ensure antigenic variation and evasion of host immunity and mediate the binding of parasite-infected RBC to the vascular endothelium (cytoadherence), to RBC (rosetting), to WBC, and sequestration of cells.^{4–6} The expression of *STEVAR* on the RBC leads to *PfEMP1*-independent binding of infected RBCs to uninfected RBCs (rosette formation), while antibodies targeting *STEVAR* in the merozoite can effectively inhibit invasion.⁷

The A, B, and H carbohydrate antigens on the surface of RBC make up the ABO blood group system, which is the most significant one in clinical practice when it comes to the relationship between malaria infection.⁸ It is one of the hereditary factors that may affect a person's vulnerability to the clinical effects of malaria.^{5,8-10} Anemia, thrombocytopenia, leukopenia, and leukocytosis are hematological abnormalities that have been associated with malaria infection and are thought to be its distinguishing feature.¹¹

Cytoadherence and rosetting contribute to the pathophysiology of malaria by obstructing blood flow, resulting in a shortage of oxygen in tissues, excessive lactate formation, and a decline in the pH of blood and tissues, which can result in severe anemia.⁵ It is characterized by hemoglobin (Hgb) levels that are below 12 g/dl for women and 13 g/dl for males.¹²

Another very common malarial consequence is thrombocytopenia, which is characterized by low PLT levels ($<150 \times 10^3$ cells/ μ L).¹³ Leukopenia and leukocytosis are also seen throughout the world, notably in Africa.^{14,15} A study carried out in Ethiopia also revealed leukopenia and leukocytosis among malaria-infected patients.¹⁶ The ABO blood group is one of the genetic factors linked to human susceptibility to severe malaria infection.⁵ Some researchers have looked at the molecular nature of the interaction between the ABO blood group and *P. falciparum* via the binding of parasitized RBC to RBC.^{5,17} Yet, and this is crucial, the ABO blood group antigens are equally expressed on the vascular endothelium and serum proteins, indicating that they may be responsible for the development of severe malaria.¹⁸

According to several studies, people with blood groups A and B are more likely to develop severe malaria than people with blood group O^{19,20} and have a higher frequency of anemia.²¹ On the other hand, a few studies revealed that people with the O blood group had the highest proportion of malaria infections, followed by those with the A blood group.^{19,22} Hematological abnormalities have been observed in malaria patients. Nevertheless, there is not much information available regarding the prevalence of anemia, thrombocytopenia, leukopenia, and leukocytosis in association with the ABO blood groups. Therefore, this study aimed to assess the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia.

Materials and Methods

Study Design, Area, and Period

A cross-sectional study was conducted in the Harar region, which is one of the ten regional states of the Federal Democratic Republic of Ethiopia, with Harar as the capital city. This region is found 526 kilometers east of Addis Ababa, the capital city of Ethiopia. According to the Harari Regional Health Bureau's 2018 annual report, the region consists of four hospitals (two of which are public and private hospitals), and ten health centers. Of those ten health centers, the study was carried out at Jinella Health Center from July 10, 2022, to January 10, 2023.

Study Participants

The study population consisted of all adult patients (aged between 18 and 65 years old) who had been confirmed to have malaria and gave their consent to take part in the study. Contrarily, individuals who were positive for infectious diseases (Human Immunodeficiency Virus (HIV), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus (HCV)), patients who were pregnant, patients who had intestinal parasites, and patients with history of chronic disease (hypertension, heart disease, and diabetes mellitus) obtained through clinical records or patient cards were excluded from the study.

Sample Size Determination and Sampling Technique

The sample size for the prevalence of thrombocytopenia among malaria-infected patients was determined using single population proportion formula considering the following assumptions: 95% confidence level, 5% margin of error, and proportion of thrombocytopenia among malaria-infected patients conducted in Ethiopia (84%).²³ This provided us with a sample size of 206. The sample size for the hematology abnormality in association with ABO blood groups was calculated in Open Epi Info 7.2.5 statistical software assuming the following assumptions: 95% confidence level, 80% power, equal unexposed to the exposed ratio (1:1), the proportion of anemia among malaria patients of A blood group (41.6%) and B blood group (22.9%).²¹ This provided a sample size of 231. The sample size calculated for the proportion

of malaria anemia among blood groups (231) was used for this study as it was greater than the calculated sample size for thrombocytopenia among malaria-infected patients. After the addition of 10% of non-responders, a total of 254 study participants were recruited using a convenience sampling technique.

Data Collection Procedure

After being written in English, the questionnaire was translated into two regional dialects (Amharic and Afan Oromo), and then back into English. Before data collection, collectors selected patients who qualified for the inclusion criteria and obtained informed consent. Socio-demographic information, clinical records, and medical history of patients were collected by administering the questionnaire under the supervision of the principal investigator.

Laboratory Sample Collection and Malaria Diagnosis

Four mL of venous blood was drawn from each patient and put into ethylene diamine tetraacetic acid (EDTA) tubes using a syringe and needle. For microscopic analysis, 1 drop (about 30 μ L) and 2 drops (about 60 μ L) of blood from each EDTA test tube were used to prepare thin and thick blood films on labeled slides, respectively. Methanol was used to fix the thin film for 10 seconds. Later, both films were stained with 10% Giemsa for 10 minutes, then carefully washed and air-dried. The slides were examined under a light microscope with an oil immersion ($\times 100$) objective.²⁴ The parasitemia level was calculated on a thick blood film using the following formula; $\text{Parasites}/\mu\text{L blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ WBC}/\mu\text{L}}{\text{Number of WBC counted}}$ ²⁵ and it was defined as high parasitemia (>10 parasites per field), moderate parasitemia (1–10 parasites per field), and low parasitemia (1–100 parasites per 100 fields).¹²

Blood Group Determination

ABO blood groups were typed using commercially available monoclonal antisera (Agape Diagnostics Ltd., India). Two drops of whole blood from an EDTA test tube were placed in each of three grease-free, clean glass slides on which the same proportion (two drops) of antisera for blood groups A, B, and Rh was applied. The blood and the antisera were mixed with an applicator stick. The slide was then tilted to detect agglutination, and the result was recorded accordingly as blood groups A+, B+, AB+, and O+, or A-, B-, AB-, and O-.²⁶

Determination of Hgb Concentration, Total WBC, and PLT Counts

The remaining blood sample in the EDTA test tube was used to determine Hgb concentration, RBC, total WBC, and PLT counts using an automated hematology analyzer UniCel DxH 800 Coulter (Beckman Coulter, Inc, Miami, FL), following the protocol of the manufacturer. Major blood cells such as Hgb, RBC, total WBC count, and PLT were recorded. To define anemia in the patients, hemoglobin values of <12 g/dl for women and <13 g/dl for men were used. Anemia was further classified as mild (Hgb 10.0–10.9 g/dl), moderate (Hgb 7–9.9 g/dl), and severe (Hgb <7 g/dl).^{27,28} A WBC count of $>10,000$ cells/ μ L was classified as leukocytosis, while leucopenia was defined as a WBC count <4000 cells/ μ L.²⁹ A PLT count $<150,000$ cells/ μ L was used to define thrombocytopenia. It was further classified as mild (PLT between 100,000 and 150,000 cells/ μ L), moderate (PLT between 50,000 and 100,000 cells/ μ L), severe (PLT $<50,000$ cells/ μ L).^{30,31}

Statistical Analysis of the Data

Following data collection, the data were edited and cleaned, and each questionnaire's completion and coding were verified. Data were entered into Epi-Data version 4.4.2.1, and all data were edited, organized, coded, exported, and analyzed using Statistical Package for Social Sciences (SPSS) version 26 statistical software. Tables, percentages, and frequencies were used to describe categorical variables. The normal distribution of continuous data was examined using the Kolmogorov–Smirnov test. Bivariable logistic regression analysis was used, and a Crude Odds Ratio (COR) with a 95% confidence interval (CI) was computed to assess the association between each independent variable and the outcome variables. Variables with a p-value of < 0.25 were considered candidates for multivariable logistic regression analysis. Model goodness-of-fit was checked by the Hosmer and Lemeshow test, and the final model was well-fitted with

the included independent variables (P value = 0.82). The final model was performed to control the confounding variables and identify the associated factors by estimating the Adjusted Odds Ratio (AOR) with a 95% CI. Statistical significance was declared at a p -value of < 0.05 .

Data Quality Control

The questionnaire was translated into the local dialects (Afan Oromo and Amharic) for data collection and then translated back into English. A week before the actual data collection, 5% of the questionnaires were pre-tested among adult patients having malaria at the Babile Health Center, which is about 24.9 kilometers from the study area. The completeness of each questionnaire was checked by the investigators daily. All laboratory activities were performed with strict adherence to standard operating procedures (SOP). The quality and expiration dates of the methanol used for thin blood fixation and Giemsa stain were checked. Giemsa was used to stain positive malaria slides to ensure the product's quality. Before deeming the slide negative, up to 100 high-power fields were viewed. An expert laboratory technologist reviewed any discrepancies in the blood film results between the two laboratory professionals. Strict adherence to the automated hematology instructions was observed. Quality checks were carried out daily using low, normal, and high control levels to track an instrument's performance over time before beginning sample analysis.

Ethical Consideration

The ethical approval was received from the Institutional Health Research Ethics Review Committee (IHRERC), College of Health and Medical Sciences, Haramaya University. Informed, voluntary, written, and signed consent was obtained before the initiation of the study from each participant and the health center head. Both the measurements and the interviews took place in private settings in different rooms. All possible identifiers were excluded from the questionnaires to protect participant confidentiality. The study was carried out in accordance with the Declaration of Helsinki.

Results

Socio-Demographic Characteristics

Of the total of 254 malaria-infected patients who took part in the study, 139 (54.7%) were male. The highest 86 (33.9%) number of participants was found to be within the age range of 18–27 years, while the least number was found to be within the age range of 58–65 years. One hundred thirty-six (53.5%) of the respondents were married, and 7 (2.8%) were widowed. The majority, 144 (56.7%) were living in rural areas, and 93 (36.6%) were able to write and read. Regarding occupational status, the highest 70 (27.5%) number of the study participants were farmers (Table 1).

Association of ABO Blood Group with Malaria Infection

The ABO blood group of all malaria patients was also examined. Accordingly, the highest prevalence of malaria infection was observed among subjects with blood groups A and B, with a prevalence of 91 (35.8%) and 77 (30.3%), respectively. While individuals with blood group AB (34 (13.4%)) were the least affected. Predominantly, 64 (70.3%) A blood group, 21 (61.8%) AB blood group, and 25 (48.1%) O blood group patients were infected with *P. falciparum* whereas the highest 36 (46.8%) of B blood group malaria patients were infected with *P. vivax*. Eleven (12.1%) A blood group patients and 5 (14.7%) AB blood group patients were infected with mixed infections. Overall, there was a statistically significant association between ABO blood groups and malaria severity ($\chi^2 = 19.575$, $p = 0.003$). Regarding the Rh factor, the majority of 226 (89.0%) malaria patients were Rh-positive. One hundred twenty-seven (88.2%), 71 (88.8%), and 28 (93.3%) malaria-infected with *P. falciparum*, *P. vivax*, and mixed infection were Rh-positive, respectively. But there was no statistically significant association between the Rh factor and malaria severity ($\chi^2 = 0.675$, $p = 0.71$). ($p = 0.71$) (Table 2).

Association of ABO Blood Groups with Anemia

The mean hemoglobin level of the study participants was $12.1\text{g/dl} \pm 2.8$ and ranged from 5.7 to 17.4 g/dl. The overall prevalence of anemia among malaria-infected adult patients was 47.2% (95% CI: 41.0–53.6). Out of this prevalence, 53

Table 1 Socio-Demographic Characteristics of the Study Participants at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N = 254)

Variables	Category	Malaria Patients N (%)
Age in years	18–27	86 (33.9)
	28–37	70 (27.6)
	38–47	41 (16.1)
	48–57	38 (15.0)
	58–65	19 (7.5)
Sex	Male	139 (54.7)
	Female	115 (45.3)
Marital Status	Single	97 (38.2)
	Married	136 (53.5)
	Divorced	14 (5.5)
	Widowed	7 (2.8)
Residence	Urban	110 (43.3)
	Rural	144 (56.7)
Educational Status	Unable to read and write	73 (28.7)
	Able to read and write	93 (36.6)
	Preparatory school	46 (18.1)
Occupational Status	College and above	42 (16.5)
	Governmental	51 (20.1)
	Merchant	46 (18.1)
	Student	42 (16.5)
	Farmer	70 (27.5)
	Housewife	38 (15.0)
	Other	7 (2.8)

Abbreviation: N, Number.

Table 2 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N=254)

Blood Group System		Number of Examined	Infection Type			χ^2 , P-value
			<i>P. falciparum</i>	<i>P. vivax</i>	Mixed	
Blood group	A	91	64 (70.3)	16 (17.6)	11 (12.1)	$\chi^2 = 19.575$ P=0.003
	B	77	34 (44.2)	36 (46.8)	7 (9.1)	
	AB	34	21 (61.8)	8 (23.5)	5 (14.7)	
	O	52	25 (48.1)	20 (38.5)	7 (13.5)	
Rh Type	Rh (+)	226	127 (56.2)	71 (31.4)	28 (12.4)	$\chi^2 = 0.675$ P=0.714
	Rh (-)	28	17 (60.7)	9 (32.1)	2 (7.1)	

Abbreviations: N, Number; *P. falciparum*, *Plasmodium falciparum*; *P. vivax*, *Plasmodium vivax*; Mixed, an infection of *P. falciparum* and *P. vivax*; Rh (+), Rh positive; Rh (-), Rh negative; χ^2 , Chi square.

(20.9%), 60 (23.6%), and 7 (2.8%) had mild, moderate, and severe anemia, respectively. Blood group A individuals (51.7%) had the highest prevalence of anemia, followed by blood group B individuals (22.5%). The lowest prevalence of anemia was 7.5% among blood group AB individuals. The most severe anemia was detected in individuals with blood group A (5 (71.4%)). Twenty-eight (46.7%) and 16 (26.7%) of the A and B blood groups, respectively, showed the highest levels of moderate anemia. The highest mild anemia appeared among 29 (54.7%) of blood group A individuals,

Table 3 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with Anemia Among Study Participants at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =120)

Blood Group System		Anemia Classification				χ^2 , P-value
		Mild	Moderate	Severe	Non-Anemic	
Blood Group	A	29(54.7)	28(46.7)	5(71.4)	29(21.6)	$\chi^2 = 30.262$ P < 0.001
	B	11(20.8)	16(26.7)	0(00)	50(37.5)	
	AB	3(5.7)	5(8.3)	1(14.3)	25(18.7)	
	O	10(18.9)	11(18.3)	1(14.3)	30(22.4)	
Rh Type	Rh (+)	47(88.7)	54(90.0)	5(71.4)	120(89.6)	$\chi^2 = 2.312$ P = 0.510
	Rh (-)	6(11.3)	6(10.0)	2(28.6)	14(10.4)	

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2 , Chi square.

and the lowest was among 3 (5.7%) of blood group AB individuals. There was a statistically significant association between the ABO blood group and the anemia status of the study participants ($p < 0.001$). The majority of mild, moderate, and severe anemias were Rh (+), with proportions of 47 (88.7%), 54 (90.0%), and 5 (71.4%), respectively (Table 3).

Association of ABO Blood Groups with Thrombocytopenia

The mean platelet value of the study participants was 140×10^3 cells/ $\mu\text{L} \pm 84.3$ and ranged from 39 to 405×10^3 cells/ μL . The overall prevalence of thrombocytopenia was 67.7% (95% CI: 61.6–73.4). The highest prevalence of thrombocytopenia was detected among individuals with blood group A (67 (39.0%)), followed by blood group B (47 (27.3%)). The lowest prevalence of thrombocytopenia was 24 (14.0%) in blood group AB individuals. Out of the total participants, 84 (33.1%), 60 (23.6%), and 28 (11.0%) had mild, moderate, and severe thrombocytopenia, respectively. The most severe thrombocytopenia was detected in individuals with blood group B (11 (39.6%)). However, the highest prevalence of moderate and mild thrombocytopenia was found among blood group A participants, with a prevalence of 22 (36.7%) and 36 (42.9%), respectively. There was no statistically significant association between the ABO blood group and thrombocytopenia in the study participants ($p = 0.319$). The majority of mild, moderate, and severe thrombocytopenia were Rh (+), with proportions of 71 (84.5%), 55 (91.7%), and 27 (96.4%), respectively (Table 4).

Association of ABO Blood Group with WBC Alteration

The mean WBC value of the study participants was 6.1×10^3 cells/ $\mu\text{L} \pm 3.1$ and ranged from 2.2 to 14.1×10^3 cells/ μL . Of the total number of study participants, 81 (31.9%) of them had leukopenia, and 33 (13.0%) of them also had leukocytosis. The highest number of leukopenia was observed among participants with blood group A (32 (39.5%)), followed by blood group O (21 (25.9%)). However, the lowest prevalence of leukopenia was 11 (13.6%) among blood

Table 4 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with Thrombocytopenia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =172)

Blood Group System		Thrombocytopenia Classification				χ^2 , P-value
		Mild	Moderate	Severe	No-Thrombocytopenia	
Blood Group	A	36(42.9)	22(36.7)	9(32.1)	24(29.3)	$\chi^2 = 10.396$ P = 0.319
	B	21(25.0)	15(25.0)	11(39.3)	30(36.6)	
	AB	9(10.9)	13(21.7)	2(7.1)	10(12.2)	
	O	18(21.4)	10(16.7)	6(21.4)	18(22.0)	
Rh Type	Rh (+)	71(84.5)	55(91.7)	27(96.4)	73(89.0)	$\chi^2 = 3.726$ P = 0.293
	Rh (-)	13(15.5)	5(8.3)	1(3.6)	9(11.0)	

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2 , Chi square.

Table 5 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with WBC Alterations Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =114)

Blood Group System		White Blood Cell Alterations			χ^2 , P-value
		Leukopenia	Leukocytosis	Normal	
ABO Blood Group	A	32(39.5)	9(27.3)	50(35.7)	$\chi^2 = 7.934$ P= 0.243
	B	17(21.0)	11(33.3)	49(35.0)	
	AB	11(13.6)	4(12.1)	19(13.6)	
	O	21(25.9)	9(27.3)	22(15.7)	
Rh Type	Rh (+)	72(88.9)	29(87.9)	125(89.3)	$\chi^2 = 0.055$ P=0.973
	Rh (-)	9(11.1%)	4(12.1)	15(10.7)	

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2 , Chi square.

group AB individuals. Seventy-two (88.9%) who had leukopenia were RH (+). Regarding leukocytosis, the highest number of leukocytosis was detected in blood group B (11 (33.3%)). However, the least leukocytosis appeared among blood group AB (4 (12.1%)) participants. Twenty-nine (87.9%) study participants who had leukocytosis were RH (+). There was no statistically significant association between the ABO blood group, Rh type, and WBC alterations (P = 0.243) (Table 5).

Factors Associated with Anemia

Bivariable and multivariable binary logistic regression analyses were performed to determine parameters associated with the anemia status of adult malaria patients. In the bi-variable binary logistic regression analysis; sex, marital status, educational status, parasitemia level, *Plasmodium species*, and blood group were significant at a p-value of <0.25 and considered candidates for multivariable logistic regression analysis. In multivariable logistic regression, sex, *Plasmodium species*, and blood group were found to have a significant association with anemia at a p-value of <0.05. The odds of being anemic among female malaria-infected patients were 3.2 times more likely (AOR = 3.18, 95% CI = 1.67, 6.04) than their male counterparts. Malaria patients infected with *P. falciparum* were 2.64 times more likely (AOR = 2.64, CI = 1.259, 5.53) to develop anemia than those infected with *P. vivax*. Blood group A malaria-infected patients were 2.75 times more likely (AOR = 2.75, CI = 1.20, 6.31) to develop anemia than blood group O patients (Table 6).

Factors Associated with Thrombocytopenia

Both bi-variable and multivariable binary logistic regression analyses were conducted to identify factors associated with thrombocytopenia in malaria-infected adult patients. In the bi-variable binary logistic regression analysis; sex, educational status, occupational status, parasitemia level, and *Plasmodium species* were significantly associated at a p-value of <0.25 and considered candidates for multivariable logistic regression analysis. In multivariable logistic regression, *Plasmodium species* and parasitemia level were found to have a significant association with thrombocytopenia at a p-value of <0.05. Malaria patients infected with *P. falciparum* were 8.03 times more likely (AOR = 8.03, CI = 3.53, 18.25) to develop thrombocytopenia than those infected with *P. vivax*. Malaria-infected patients with a high parasitemia level were 4.40 times more likely (AOR = 4.40, CI = 1.57, 12.32) to develop thrombocytopenia than malaria-infected patients with a low parasitemia level (Table 7).

Discussion

Hematological abnormalities are one of the most common complications of malaria infection, along with malarial anemia, thrombocytopenia, and leukocytosis or leucopenia.¹² The findings of this study assessed the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia. Accordingly, the hemoglobin test result showed that 47.2% of study participants were anemic. Being female, having an “A” blood group, and being infected with *P. falciparum* were identified as associated factors of anemia among malaria-infected patients in association with ABO blood groups. On the other hand, A PLT count <150,

Table 6 Logistic Regression Analysis of Associated Factors with Anemia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N=254)

Variables	Category	Anemia Status		95% CI	
		Yes N (%)	No N (%)	COR	AOR
Age in years	18–27	43(50.0)	43(50)	1	1
	28–37	35(50.0)	35(50.0)	1.000(0.532, 1.880)	1.164(0.500, 2.713)
	38–47	18(43.9)	23(56.1)	0.783(0.371, 1.653)	0.755 (0.275, 2.076)
	48–57	16(42.1)	22(57.9)	0.727(0.337, 1.571)	0.666 (0.245, 1.811)
	58–65	8(42.1)	11(57.9)	0.727(0.266, 1.985)	0.370 (0.110, 1.241)
Sex	Female	67(58.3)	48(41.7)	2.265(1.368, 3.751)	3.177 (1.671, 6.038)*
	Male	53(38.1)	86(61.9)	1	1
Marital Status	Single	45(46.4)	52(53.6)	1	1
	Married	62(45.6)	74(54.4)	0.968 (0.574, 1.633)	0.717 (0.378, 1.363)
	Divorced	9(64.3)	5(35.7)	2.080 (0.650, 6.660)	1.740 (0.438, 6.913)
	Widowed	4(57.1)	3(42.9)	1.541(0.327, 7.254)	1.294 (0.191, 8.754)
Residence	Urban	54(49.1)	56(50.9)	1	1
	Rural	66(45.8)	78 (54.2)	0.877 (0.534, 1.443)	0.758 (0.394, 1.461)
Educational Status	Unable to read and write	41(56.2)	32(43.8)	1	1
	Able to read and write	41(44.1)	52(55.9)	0.615(0.332, 1.141)	0.563 (0.244, 1.301)
	Preparatory School	19 (41.3)	27(58.7)	0.549(0.260, 1.159)	0.350 (0.087, 1.403)
	College and above	19(45.2)	23 (54.8)	0.645(0.300, 1.383)	0.500(0.163, 1.534)
Occupational Status	Governmental	26(51.0)	25(49.0)	1	1
	Merchant	21(45.7)	25(54.3)	0.808 (0.363, 1.795)	0.566 (0.172, 1.869)
	Student	19(45.2)	23 (54.8)	0.794 (0.350, 1.802)	1.724 (0.374, 7.945)
	Farmer	32 (45.7)	38(54.3)	0.810 (0.393, 1.668)	0.914 (0.315, 2.647)
	Housewife	18 (47.4)	20(52.6)	0.865 (0.373, 2.006)	0.571(0.182, 1.794)
	Other	4(57.1)	3(42.9)	1.282 (0.260, 6.315)	1.035 (0.124, 8.665)
<i>Plasmodium Species</i>	<i>P. falciparum</i>	83 (57.6)	61(42.4)	2.671(1.512, 4.719)	2.638 (1.259, 5.527)*
	Mixed	10 (33.3)	20 (66.7)	0.981(0.403, 2.389)	0.597 (0.209, 1.709)
	<i>P. vivax</i>	27(33.8)	53(66.3)	1	1
Parasitemia Level	High	23(46.0)	27(54.0)	0.681(0.348, 1.336)	0.572 (0. 252, 1.296)
	Moderate	37(38.5)	59 (61.5)	0.502(0.287, 0.878)	0.576 (0.283, 1.172)
	Low	60 (55.6)	48 (44.4)	1	1
Blood Group	A	62 (68.1)	29(31.9)	2.915 (1.440, 5.900)	2.746 (1.195, 6.309)*
	B	27(35.1)	50(64.9)	0.736 (0.358, 1.516)	0.862 (0.371, 2.003)
	AB	9(26.5)	25(73.5)	0.491(0.192, 1.256)	0.481(0.166, 1.396)
	O	22(42.3)	30(57.7)	1	1
Rh Type	Rh (+)	106(46.9)	120(53.1)	1	1
	Rh (-)	14(50.0)	14(50.0)	1.132 (0.516, 2.483)	0.811 (0.310, 2.123)

Note: *Significantly associated at P value of <0.05.

Abbreviations: N, Number; *P. falciparum*, *Plasmodium falciparum*; *P. vivax*, *Plasmodium vivax*; Mixed, An infection of *P. falciparum* and *P. vivax*; COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; Rh (+), Rh positive; Rh (-), Rh negative.

000 cells/ μ L was used to define thrombocytopenia, and 67.7% of study participants had thrombocytopenia. Parasitemia level and *P. falciparum* were identified as associated factors of thrombocytopenia among malaria-infected patients in association with ABO blood groups.

The present study showed significantly varied degrees of malaria infection among the ABO blood groups ($P = 0.003$). The highest (35.8%) malaria infection was observed among individuals with A (35.8%), followed by B (30.3%), and O (20.5%) blood groups, while individuals with AB (13.4%) blood groups were the least affected. This result is in agreement with the findings done in Sri Lanka,³² Sudan,³³ and across different corners of Ethiopia.^{19,22,34} However, this study was contrary to the findings conducted in China,³⁵ Burkina Faso,³⁶ Ghana³⁷ and Nigeria³⁸ which reported malaria infection was generally higher in O blood groups than other blood groups. This discrepancy in results could due to

Table 7 Logistic Regression Analysis of Associated Factors with Thrombocytopenia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (n = 254)

Variables	Category	Thrombocytopenia		95% CI	
		Yes N (%)	No N (%)	COR	AOR
Age in years	18–27	53(61.6)	33(38.4)	0.574 (0.189, 1.740)	0.996(0.271, 3.663)
	28–37	53(75.7)	17(24.3)	1.113(0.350, 3.545)	3.565(0.889, 14.291)
	38–47	28(68.3)	13(31.7)	0.769(0.228, 2.592)	2.348(0.528, 10.435)
	48–57	24(63.2)	14(36.8)	0.612(0.182, 2.064)	1.846(0.431, 7.915)
	58–65	14(73.7)	5(26.3)	1	1
Sex	Female	84(73.0)	31(27.0)	1.570(0.918, 2.687)	1.723(0.869, 3.416)
	Male	88(63.3)	51(36.7)	1	1
Marital Status	Single	69(71.1)	28(28.9)	1	1
	Married	91(66.9)	45(33.1)	0.821(0.466, 1.445)	0.572(0.283, 1.157)
	Divorced	8(57.1)	6(42.9)	0.541(0.172, 1.702)	0.289(0.074, 1.133)
	Widowed	4(57.1%)	3(42.9)	0.541(0.114, 2.575)	0.600(0.091, 3.977)
Residence	Urban	75(68.2)	35(31.8)	1	1
	Rural	97(67.4)	47(32.6)	0.963(0.566, 1.638)	0.928(0.462, 1.865)
Educational Status	Unable to read and write	52(71.2)	21(28.8)	1	1
	Able to read and write	64(68.8)	29(31.2)	0.891(0.456, 1.742)	0.743(0.303, 1.820)
	Preparatory School	28(60.9)	18(39.1)	0.628(0.288, 1.370)	1.147(0.239, 5.504)
	College and above	28(66.7)	14(33.3)	0.808(0.357, 1.829)	0.907(0.279, 2.952)
Occupational Status	Governmental	37(72.5)	14(27.5)	1	1
	Merchant	35(76.1)	11(23.9)	1.204(0.482, 3.006)	1.196(0.327, 4.380)
	Student	23(54.8)	19(45.2)	0.458(0.193, 1.087)	0.638(0.132, 3.089)
	Farmer	40(57.1)	30(42.9)	0.505(0.232, 1.096)	0.613(0.198, 1.904)
	Housewife	31(81.6)	7(18.4)	1.676(0.601, 4.671)	1.176(0.327, 4.229)
	Other	6(85.7)	1(14.3)	2.270(0.250, 20.582)	1.837(0.142, 23.702)
<i>Plasmodium</i> Species	<i>P. falciparum</i>	119(82.6)	25(17.4)	6.120(3.301, 11.346)	8.030(3.534, 18.248)*
	Mixed	18(60.0)	12(40.0)	1.929 (0.821, 4.529)	2.049(0.742, 5.656)
	<i>P. vivax</i>	35(43.8)	45(56.3)	1	1
Parasitemia Level	High	42(84.0)	8(16.0)	2.736(1.165, 6.428)	4.396(1.569, 12.318)*
	Moderate	59(61.5)	37(38.5)	0.831(0.469, 1.472)	1.251(0.582, 2.693)
	Low	71(65.7)	37(34.3)	1	1
Blood group	A	67(73.6)	24(26.4)	1.478(0.707, 3.090)	0.983(0.403, 2.402)
	B	47(61.0)	30(39.0)	0.829(0.399, 1.725)	1.040(0.428, 2.526)
	AB	24(70.6)	10(29.4)	1.271(0.500, 3.230)	0.968(0.317, 2.953)
	O	34(65.4)	18(34.6)	1	1
Rh Type	Rh (+)	153(67.7)	73(32.3)	1	1
	Rh (-)	19(67.9)	9(32.1)	1.007(0.435, 2.335)	1.187(0.432, 3.263)

Note: *Significantly associated at P value of <0.05.

Abbreviations: N, Number; *P. falciparum*, *Plasmodium falciparum*; *P. vivax*, *Plasmodium vivax*; Mixed, an infection of *P. falciparum* and *P. vivax*; COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; Rh (+), Rh positive; Rh (-), Rh negative.

mutations in the genetic mechanisms (A, B, and H carbohydrate antigens), which can regulate protein activities during infection and antibodies against the malaria parasite.^{39,40}

A higher proportion of malaria was found to be Rh-positive (89.0%) individuals than Rh-negative individuals, even though no statistically significant association was seen as similar studies reported in India,⁴¹ Senegal,⁴² and Nigeria.^{43,44} It is uncertain how the Rh- blood group could work as a protective or risk factor for malaria. However, the Rh-blood group changes the parasites' adhesion properties to the RBC membrane and sequesters itself in less suitable areas for malaria infection.⁴⁵

In this study, the overall anemia prevalence was 47.2%. Blood group A malaria-infected patients were 2.75 times more likely to develop anemia than blood group O patients. This finding was supported by some studies conducted in

India,⁴⁶ Nigeria,⁴⁷ and Ethiopia.²¹ The high risk of anemia observed in malaria patients with blood group A might be attributed to the presence of specific receptors (band 3 and glycophorin A) and glycosylated adhesive molecules, which assist in the fast invasion of RBCs by *P. falciparum*.⁴⁸

Whereas the current study disagreed with the results of the study conducted in Colombia⁴⁹ that reported a more frequent occurrence of severe anemia among individuals with blood group O. Additionally, blood group AB subjects had the least anemia, in contrast to a study conducted in Cameroon,⁵⁰ which reported the most anemic blood group. This inconsistency might be attributed to the presence of A and B antigens on the surface of RBC; cytoadherence, hence rosetting and sequestration, is increased in individuals with blood groups A and B.⁵¹ Another possible explanation may be due to the presence of sialic acid and galactose in the carbohydrate structure of glycophorin A or B, which are the major requisites for the entrance of *P. falciparum* merozoites into human RBC.⁵²

The odds of being anemic among female malaria-infected patients were 3.2 times higher than those of their male counterparts. This finding was supported by a study done in India,⁵³ and Cameroon.⁵⁴ The possible explanation might be due to chronic blood loss and abnormal menstrual function among female participants.⁵⁵ Malaria patients infected with *P. falciparum* were 2.64 times more likely to develop anemia than those infected with *P. vivax*. This result was in agreement with studies conducted in Colombia⁴⁹ and Ethiopia.²¹ But in contrast with a study conducted in Australia.⁵⁶ This discordant result of anemia in *plasmodium species* might be multifactorial and incompletely understood. But in *P. falciparum*, RBC sequestration reduces the proportion of parasitized RBC that traverse the spleen, while the increased deformability of infected RBC in *vivax* malaria may limit the proportion of RBC that are removed during passage through the splenic microcirculation.^{57,58}

In this study, the overall prevalence of thrombocytopenia was 67.7%. The highest prevalence of thrombocytopenia was detected among study participants belonging to blood group A (39.0%), followed by blood group B (27.3%), while its distribution was lower in subjects with AB (14.0%) blood groups. The most severe thrombocytopenia was detected in individuals with blood group B (39.6%). However, the highest prevalence of moderate and mild thrombocytopenia was found among blood group A participants, with a prevalence of 22 (36.7%) and 36 (42.9%), respectively. According to the current study, no significant association was found ($p = 0.319$) between the blood group and thrombocytopenia, and all ABO blood groups showed similar falls in platelet levels, suggesting that thrombocytopenia was not affected by the ABO blood groups. Studies from India,⁵⁹ and Pakistan⁶⁰ showed similar findings.

Patients with *P. falciparum* malaria were 8.03 times more likely to develop thrombocytopenia than those with *P. vivax* malaria. This result was supported by studies conducted in India,⁶¹ Indonesia,⁶² Pakistan,⁶³ and Sudan.⁶⁴ The pathogenic mechanisms by which platelets mediate disease severity in patients with *P. falciparum* malaria remain to be delineated. However, a study has shown that the platelets of *P. falciparum* patients express Toll-like receptors (TLRs), which release prepared inflammatory mediators.⁶⁵ Another reason for thrombocytopenia in malaria infections, particularly those brought on by *P. falciparum*, could be the immune-mediated degradation of circulating platelets.⁶⁶

Malaria-infected patients with a high parasitemia level were 4.40 times more likely to develop thrombocytopenia than malaria-infected patients with a low parasitemia level. This result was supported by studies done in Thailand,⁶⁷ Senegal,⁶⁸ and Sudan.⁶⁴ Evidence suggests that malaria thrombocytopenia at high parasitemia was found to significantly lower the PLT count.⁶⁹ As malaria parasitemia increases, platelets play a critical role in enabling the cytoadherence of malaria-infected erythrocytes (IEs) to ECs, and Von Willebrand factor (VWF) then promotes this platelet-mediated IE cytoadherence during the malaria infection.⁷⁰ Finally, platelets enhance IE clumping and sequestration, which further contributes to its decrease during the infection.⁷¹

Changes in WBC count are less dramatic than in other blood cell series and have been controversial. Of the total number of study participants, 81 (31.9%) had leukopenia. This study was in accordance with the studies conducted in Colombia⁷² and Pakistan.⁷³ This could be a result of the sequestration of leukocytes, accelerated destruction, or decreased production.⁷⁴ The highest number of leukopenia was observed among blood group A (39.5%), followed by blood group O study participants (25.9%), in contrast with the studies conducted in India⁵⁹ and Pakistan⁶⁰ which were higher in B and AB blood groups, respectively. This discrepancy in results across different studies might be due to the methods they used for the determination of WBC counts and statistical tests that assume Gaussian distributions of data.⁷⁵

Regarding leukocytosis, 33 (13.0%) of the study participants had leukocytosis. The same results were reported from Burkina Faso⁷⁶ and Thailand.⁶⁷ The highest number of leukocytosis was detected in blood group B (33.3%), similar to

a study conducted in Pakistan.⁶⁰ This might be attributed to the increased production of leukocytes at the onset of an infection to engulf the invading parasite.⁷⁷ The ABO blood group and WBC alterations among malaria-infected patients did not have a statistically significant correlation.

Strengths and Limitations of the Study

The study's strength is that all hematological abnormalities were detected. However, due to its cross-sectional nature, the study is unable to demonstrate the temporal relationship between the hematological abnormalities and the identified related factors. Additionally, the study did not apply any reliable assays, like PCR, that can distinguish distinct malaria parasites with comparable characteristics.

Conclusion

Of the examined malaria-infected adult patients, almost half, more than half, one-third, and almost one-eighth of the patients had anemia, thrombocytopenia, leukopenia, and leukocytosis, respectively. There was a significant association between the ABO blood groups and susceptibility to malaria-related anemia. Being female, having an "A" blood group, and being infected with *P. falciparum* were identified as associated factors of anemia among malaria-infected patients about ABO blood groups. On the other hand, parasitemia level and *P. falciparum* were identified as associated factors of thrombocytopenia among malaria-infected patients in relation to ABO blood groups. There was no significant association between blood groups and susceptibility to malaria-developing thrombocytopenia, leukopenia, or leukocytosis. Therefore, there should be routine screening for anemia, thrombocytopenia, and WBC alterations for all malaria patients.

Abbreviations

BF, Blood Film; EDTA, Ethylene Diamine Tetra Acetic Acid; ECs, endothelial cells; Hgb, Hemoglobin; ICAM-1, Intravascular cell adhesion molecule-1; IEs, Infected erythrocytes; RBC, Red Blood Cell; *P. Falciparum*, *Plasmodium Falciparum*; PLT, Platelet; *P. vivax*, *Plasmodium Vivax*; VWF, Von Willebrand factor; WBC, White Blood Cell.

Acknowledgments

The authors are grateful to all Jinella Health Center staff members, the data collectors, supervisors, study participants, and questionnaire translators for their cooperation.

Author Contributions

All authors significantly contributed to the work that was published by participating in the ideation, study design, execution, data collection, analysis, and interpretation processes as well as in the writing, editing, and review of the article. All authors have agreed on the approval of the final manuscript to be published in the current journal and to be accountable for all aspects of the work.

Funding

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. World Health Organization. *Eliminating Malaria*. World Health Organization; 2016.
2. Mohandas N, An X. Malaria and human red blood cells. *Med Microbiol Immunol*. 2012;201(4):593–598. doi:10.1007/s00430-012-0272-z
3. Cox D, McConkey S. The role of platelets in the pathogenesis of cerebral malaria. *Cell Mol Life Sci*. 2010;67(4):557–568. doi:10.1007/s00018-009-0211-3
4. Plewes K, Turner G, Dondorp A. Pathophysiology, clinical presentation, and treatment of coma and acute kidney injury complicating falciparum malaria. *Curr Opin Infect Dis*. 2018;31(1):69. doi:10.1097/QCO.0000000000000419

5. Wahlgren M, Goel S, Akhouri RR. Variant surface antigens of *Plasmodium falciparum* and their roles in severe malaria. *Nat Rev Microbiol*. 2017;15(8):479–491. doi:10.1038/nrmicro.2017.47
6. Yam XY, Niang M, Madnani KG, Preiser PR. Three is a crowd—new insights into rosetting in *Plasmodium falciparum*. *Trends Parasitol*. 2017;33(4):309–320. doi:10.1016/j.pt.2016.12.012
7. Niang M, Bei AK, Madnani KG, et al. STEVOR is a *Plasmodium falciparum* erythrocyte binding protein that mediates merozoite invasion and rosetting. *Cell Host Microbe*. 2014;16(1):81–93. doi:10.1016/j.chom.2014.06.004
8. Cserti M, Dzik H. The ABO blood group system and *Plasmodium falciparum* malaria. *Blood*. 2007;110(7):2250–2258.
9. Loscertales M, Owens S, O'Donnell J, Bunn J, Bosch-Capblanch X, Brabin B. ABO blood group phenotypes and *Plasmodium falciparum* malaria: unlocking a pivotal mechanism. *Adv Parasitol*. 2007;65:1–50.
10. Driss A, Hibbert JM, Wilson NO, Iqbal SA, Adamkiewicz TV, Stiles JK. Genetic polymorphisms linked to susceptibility to malaria. *Malar J*. 2011;10(1):1–10. doi:10.1186/1475-2875-10-271
11. Das B, Ganguly R, Khuntia H, Bal M, Ranjit M. Hematological changes in severe *P. falciparum* malaria. *Int J Curr Microbiol Appl Sci*. 2017;6(6):1733–1739. doi:10.20546/ijemas.2017.606.201
12. Al-Salahy M, Shnawa B, Abed G, Mandour A, Al-Ezzi A. Parasitaemia and its relation to hematological parameters and liver function among patients malaria in Abs, Hajjah, Northwest Yemen. *Interdiscip Perspect Infect Dis*. 2016;2016:1–5. doi:10.1155/2016/5954394
13. Bayleyegn B, Asrie F, Yalaw A, Woldu B, Gonzalez Salazar F. Role of platelet indices as a potential marker for malaria severity. *J Parasitol Res*. 2021;2021:1–8. doi:10.1155/2021/5531091
14. D'souza JJ, Jayaprakash C, D'souza P, Abraham S, Suresh S, Shrinath M. Comparative hematological changes in malarial infection by *P. vivax* and *P. falciparum*: observations from the endemic region of Mangalore, India. *Int J Appl Res*. 2017;3(6):179–183.
15. Okafor UE, Tsoka-Gwegweni JM, Bibirigea A, Irimie A, Tomuleasa C. Parasitaemia and haematological changes in malaria-infected refugees in South Africa. *S Afr Med J*. 2016;106(4):413–416. doi:10.7196/SAMJ.2016.v106i4.9758
16. Sirak S, Fola A, Worku L, Biadgo B. Malaria parasitemia and its association with lipid and hematological parameters among malaria-infected patients attending at Metema Hospital, Northwest Ethiopia. *Pathol Lab Med Int*. 2016;8:43–50. doi:10.2147/PLMI.S118946
17. Goel S, Palmkvist M, Moll K, et al. RIFINs are adhesins implicated in severe *Plasmodium falciparum* malaria. *Nat Med*. 2015;21(4):314–317. doi:10.1038/nm.3812
18. Needs M. Human blood groups (3rd edn). *Br J Biomed Sci*. 2013;70(3):131.
19. Tadesse H, Tadesse K. Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia. *J Vector Borne Dis*. 2013;50(4):292.
20. Gayathri B, Harendra K, Gomathi N, Jeevan S, Reethesh R. Relationship between ABO blood groups and malaria with clinical outcome in rural area of South India. *Glob J Med Public Health*. 2013;2(2):1–7.
21. Tazebew B, Munshea A, Nibret E. Prevalence and association of malaria with ABO blood group and hemoglobin level in individuals visiting Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia: a cross-sectional study. *Parasitol Res*. 2021;120(5):1821–1835. doi:10.1007/s00436-021-07093-z
22. Zerihun T, Degarege A, Erko B. Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pac J Trop Biomed*. 2011;1(4):289–294. doi:10.1016/S2221-1691(11)60045-2
23. Awoke N, Arota A. Profiles of hematological parameters in *Plasmodium falciparum* and *Plasmodium vivax* malaria patients attending Tercha General Hospital, Dawuro Zone, South Ethiopia. *Infect Drug Resist*. 2019;12:521. doi:10.2147/IDR.S184489
24. World Health Organization, & UNICEF. *Microscopy for the Detection, Identification and Quantification of Malaria Parasites on Stained Thick and Thin Blood Films in Research Settings: Procedure: Methods Manual*. World Health Organization; 2015.
25. World Health Organization. *Malaria Parasite Counting; Malaria Microscopy Standard Operating Procedures*. World Health Organization; 2016. Available from: [WHO/HTM/GMP/MM/SOP/2016.09](https://www.who.int/publications/malaria/gmp/mm/sop/2016.09). Accessed August 12, 2023.
26. Otajevwo F. Prevalence of malaria parasitaemia and its association with ABO blood grouping among students of Igbiniedion University Okada, Nigeria. *Br J Med Med Res*. 2013;3(4):1164–1177. doi:10.9734/BJMMR/2013/1745
27. Lanier J, Park J, Callahan R. Anemia in older adults. *Am Fam Physician*. 2018;98(7):437–442.
28. Murphy J. *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. *Vitamin and Mineral Nutrition Information System*. Geneva: World Health Organization; 2011.
29. Ngum NH, Fakeh NB, Lem AE, Mahamat O. Prevalence of malaria and associated clinical manifestations and myeloperoxidase amongst populations living in different altitudes of Mezam division, North West Region, Cameroon. *Malar J*. 2023;22(1):1–14. doi:10.1186/s12936-022-04438-6
30. Izak M, Bussell JB. Management of thrombocytopenia. *FI000Prime Rep*. 2014;6. doi:10.12703/P6-6
31. Mangrio GM, Memon MA, Shaikh MA, Memon HNA, Arwani S, Shah SZA. Vivax malaria: frequency, severity of thrombocytopenia and variation in Red Cell Distribution Width (RDW). *Prof MedJ*. 2015;22(05):559–564. doi:10.29309/TPMJ/2015.22.05.1267
32. Pathirana S, Alles H, Bandara S, et al. ABO-blood-group types and protection against severe, *Plasmodium falciparum* malaria. *Ann Trop Med Parasitol*. 2005;99(2):119–124. doi:10.1179/136485905X19946
33. Satti SMA. *Association of ABO and Rh Blood Groups with Malaria Infection in 24 Algorashi Area in Aljazeera State-Sudan*. Sudan University of Science & Technology; 2017.
34. Tekeste Z, Petros B. The ABO blood group and *Plasmodium falciparum* malaria in Awash, Metehara and Ziway areas, Ethiopia. *Malar J*. 2010;9(1):1–4. doi:10.1186/1475-2875-9-280
35. Zhang X, Yang M, Zhao H, Hu J, Li L. Relationship between malaria and ABO blood types in East China. *Biomed Res Int*. 2017;2017:1–3. doi:10.1155/2017/8163762
36. Bougouma EC, Ouedraogo A, Sirima SB. *The ABO Blood Group System and Plasmodium Falciparum (Pf) Infection in Three Ethnic Groups Living in the Stable and Seasonal Malaria Transmission Areas of Burkina Faso (BF)*. IntechOpen; 2022.
37. Osisiogu EU, Agyapong GA, Mahmoud FC, Waqas FB, Appiah C, Nikoi CN. Prevalence of malaria among ABO blood groups in Ghana: a Case Study of Adentan Municipality. *Int J Pathog Res*. 2023;12(1):21–29. doi:10.9734/ijpr/2023/v12i1217
38. Tela I, Modibbo M, Adamu L, Taura M. Prevalence of malaria infection among ABO blood groups in Jama'are, Nigeria. *RA J ApplRes*. 2015;1(7):255–262.

39. Dolo A, Modiano D, Maiga B, et al. Difference in susceptibility to malaria between two sympatric ethnic groups in Mali. *Am J Trop Med Hyg.* 2005;72(3):243–248. doi:10.4269/ajtmh.2005.72.243
40. Seyoum S, Dagne K. ABO and rhesus blood-type frequencies in data from hospitals and the Red Cross in Ethiopia. *Ethiop Med J.* 1985;23(1):1–6.
41. Singh G, Urhekar A, Singh R. A study on correlation of malaria infection with A, B, O, RH blood group system. *J Parasitol Vector Biol.* 2015;7(4):67–73.
42. Wotodjo AN, Richard V, Boyer S, et al. The implication of long-lasting insecticide-treated net use in the resurgence of malaria morbidity in a Senegal malaria endemic village in 2010–2011. *Parasit Vectors.* 2015;8(1):1–11. doi:10.1186/s13071-015-0871-9
43. Abah A, Grey A, Onoja H. Plasmodium malaria and ABO blood group among blood donors in Yenegoa, Bayelsa State, Nigeria. *J Prim Health Care.* 2016;6(4):1–4.
44. Ito E, Egwunyenga A, Ake J. Prevalence of malaria and human blood factors among patients in Ethiopia East, Delta State, Nigeria. *Int J Med Biomed Res.* 2014;3(3):191–201. doi:10.14194/ijmbr.3.3.7
45. Rattanapan Y, Duangchan T, Wangdi K, Mahittikorn A, Kotepui M. Association between rhesus blood groups and malaria infection: a systematic review and meta-analysis. *Trop Med Infect Dis.* 2023;8(4):190. doi:10.3390/tropicalmed8040190
46. Ramalingam L, Raghavan GV. Association between blood groups and blood hemoglobin levels in rural population of Kanchipuram district of Tamil Nadu. *Natl J Physiol Pharm Pharmacol.* 2020;10(6):495.
47. Mbah JO, Njoku O, Nnachi AU, Nnachi IA, Nwinyimagu AJ. Incidence of antenatal malaria parasitaemia and the effect on the haemoglobin profile of pregnant women in Enugu East Local Government Area, Enugu, Nigeria. *Am J Epidemiol Infect Dis.* 2015;3(5):88–94.
48. Degarege A, Medhin G, Anmut A, Legess M, Erko B. Association of ABO blood group and P. falciparum malaria related outcomes: a cross-sectional study in Ethiopia. *Acta Trop.* 2012;123(3):164–169. doi:10.1016/j.actatropica.2012.04.012
49. Herrera AM, Montoya LP, Arboleda M, Ortiz LF. Association of severe malaria with ABO-blood group types in an endemic zone of Colombia. *CES Med.* 2009;23(2):7–14.
50. Kwentí TE, Kwentí TDB. Anaemia and its association with month and blood phenotype in blood donors in Fako division, Cameroon. *BMC Hematol.* 2016;16:1–7. doi:10.1186/s12878-016-0070-8
51. Afoakwah R, Aubyn E, Prah J, Nwaefuna EK, Boampong JN. Relative susceptibilities of ABO blood groups to Plasmodium falciparum malaria in Ghana. *Adv Hematol.* 2016;2016:1–4. doi:10.1155/2016/5368793
52. Abegaz SB, Erg n S. Human ABO blood groups and their associations with different diseases. *Biomed Res Int.* 2021;2021:1–9. doi:10.1155/2021/6629060
53. Shankar H, Singh MP, Hussain SSA, Phookan S, Singh K, Mishra N. Epidemiology of malaria and anemia in high and low malaria-endemic north-eastern districts of India. *Front Public Health.* 2022; 2022:10.
54. Sumbele IUN, Sama SO, Kimbi HK, Taiwe GS. Malaria, moderate to severe anaemia, and malarial anaemia in children at presentation to hospital in the Mount Cameroon area: a cross-sectional study. *Anemia.* 2016;2016:1–12. doi:10.1155/2016/5725634
55. Kaur IP, Kaur S. A comparison of nutritional profile and prevalence of anemia among rural girls and boys. *J Exerc Sci Physiother.* 2011;7(1):11–18. doi:10.18376/2011/v7i1/67618
56. Douglas NM, Anstey NM, Buffet PA, et al. The anaemia of Plasmodium vivax malaria. *Malar J.* 2012;11(1):1–14. doi:10.1186/1475-2875-11-135
57. Dondorp AM, Angus B, Chotivanich K, et al. Red blood cell deformability as a predictor of anemia in severe falciparum malaria. *Am J Trop Med Hyg.* 1999;60(5):733–737. doi:10.4269/ajtmh.1999.60.733
58. Suwanarusk R, Cooke BM, Dondorp AM, et al. The deformability of red blood cells parasitized by Plasmodium falciparum and P. vivax. *J Infect Dis.* 2004;189(2):190–194. doi:10.1086/380468
59. Deepa AV, Rameshkumar K, Ross C. ABO blood groups and malaria related clinical outcome. *J Vector Borne Dis.* 2011;48(1):7–11.
60. Burhan H, Hasan AS, Mansur-ul-Haque S, Zaidi G, Shaikh T, Zia A. Association between blood group and susceptibility to malaria and its effects on platelets, TLC, and Hb. *J Infect Dev Ctries.* 2016;10(10):1124–1128. doi:10.3855/jidc.6828
61. Gill MK, Makkar M, Bhat S, Kaur T, Jain K, Dhir G. Thrombocytopenia in malaria and its correlation with different types of malaria. *Ann Trop Med Public Health.* 2013;6(2). doi:10.4103/1755-6783.116521
62. Dini S, Douglas NM, Poespoprodjo JR, et al. The risk of morbidity and mortality following recurrent malaria in Papua, Indonesia: a retrospective cohort study. *BMC Med.* 2020;18:1–12. doi:10.1186/s12916-020-1497-0
63. Khan SJ, Abbass Y, Marwat MA. Thrombocytopenia as an indicator of malaria in adult population. *Malar Res Treat.* 2012;2012:1–4. doi:10.1155/2012/405981
64. Elnaim EG, Amer S, Abdalmanan M, et al. Association of thrombocytopenia, urine malaria antigens, and blood groups with malaria parasite density among Sudanese malaria patients at Sharg Al-Nile District in Khartoum State. *Int J Adv Res Biol Sci.* 2020;7(3):17–22.
65. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood.* 2014;123(18):2759–2767.
66. Pain A, Ferguson DJ, Kai O, et al. Platelet-mediated clumping of Plasmodium falciparum-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc Natl Acad Sci.* 2001;98(4):1805–1810. doi:10.1073/pnas.98.4.1805
67. Kotepui M, Piwklam D, PhunPhuech B, Piwklam N, Chupeerach C, Duangmano S. Effects of malaria parasite density on blood cell parameters. *PLoS One.* 2015;10(3):e0121057. doi:10.1371/journal.pone.0121057
68. Sylla K, Tine R, Sow D, et al. Anemia, thrombocytopenia, and changes in biochemical parameters occurring in patients with uncomplicated Plasmodium falciparum malaria: data analysis from antimalarial efficacy-randomized trials in Dakar and Kaolack Regions, Senegal. *J Parasitol Res.* 2022;2022. doi:10.1155/2022/1635791
69. Kochar D, Kochar S, Agrawal R, et al. The changing spectrum of severe falciparum malaria: a clinical study from Bikaner (northwest India). *J Vector Borne Dis.* 2006;43(3):104.
70. O'Sullivan JM, Preston RJ, O'Regan N, O'Donnell JS. Emerging roles for hemostatic dysfunction in malaria pathogenesis. *Blood.* 2016;127(19):2281–2288.
71. McMorran BJ, Marshall VM, de Graaf C, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science.* 2009;323(5915):797–800. doi:10.1126/science.1166296
72. Tobón-Castaño A, Mesa-Echeverry E, Miranda-Arboleda AF. Leukogram profile and clinical status in vivax and falciparum malaria patients from Colombia. *J Trop Med.* 2015;2015:796182. doi:10.1155/2015/796182

73. Ullah I, Ali MU, Ali S, Rafiq A, Sattar Z, Hussain S. Hematological Profile of Patients Having Malaria-positive Peripheral Blood Smears: A Cross-sectional Study at a Diagnostic Research Center in Khyber Pakhtunkhwa, Pakistan. *Cureus*. 2018;10(9):1–12. doi:10.7759/cureus.3376
74. Okeke OP, Imakwu CA, Eyo JE, Okafor FC. Effects of childhood malaria on the biochemical and haematological profiles of infected children in Anambra State, Nigeria. *Int J Trop Dis*. 2016;19(3):1–14.
75. McKenzie FE, Prudhomme WA, Magill AJ, et al. White blood cell counts and malaria. *J Infect Dis*. 2005;192(2):323–330. doi:10.1086/431152
76. Modiano D, Sirima BS, Konaté A, Sanou I, Sawadogo A. Leucocytosis in severe malaria. *Trans R Soc Trop Med Hyg*. 2001;95(2):175–176. doi:10.1016/S0035-9203(01)90152-X
77. Kayode O, Kayode A, Awonuga O. Status of selected hematological and biochemical parameters in malaria and malaria-typhoid co-infection. *J Biol Sci*. 2011;11(5):367–373. doi:10.3923/jbs.2011.367.373

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Effect of *Moringa oleifera* Leaf Powder on Hematological Profile of Male Wistar Rats

Titing Nurhayati, Muhammad Irfan Fathoni, Siti Nur Fatimah, Vita Murniati Tarawan, Hanna Goenawan & Resti Gradia Dwiwina

To cite this article: Titing Nurhayati, Muhammad Irfan Fathoni, Siti Nur Fatimah, Vita Murniati Tarawan, Hanna Goenawan & Resti Gradia Dwiwina (2023) Effect of *Moringa oleifera* Leaf Powder on Hematological Profile of Male Wistar Rats, Journal of Blood Medicine, , 477-485, DOI: [10.2147/JBM.S407884](https://doi.org/10.2147/JBM.S407884)

To link to this article: <https://doi.org/10.2147/JBM.S407884>



© 2023 Nurhayati et al.



Published online: 23 Aug 2023.



Submit your article to this journal



Article views: 647



View related articles



View Crossmark data

Effect of *Moringa oleifera* Leaf Powder on Hematological Profile of Male Wistar Rats

Titing Nurhayati^{1,*}, Muhammad Irfan Fathoni^{2,*}, Siti Nur Fatimah^{3,*}, Vita Murniati Tarawan¹, Hanna Goenawan¹, Resti Gradia Dwiwina¹

¹Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; ²Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; ³Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

*These authors contributed equally to this work

Correspondence: Titing Nurhayati, Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM 21, Bandung, West Java, 45363, Indonesia, Tel/Fax +62 85 222221149, Email titing.nurhayati@unpad.ac.id

Background: Indonesia is a country with high biodiversity of more than 20,000 plant species, and 35% of them are identified as having health benefits. *Moringa oleifera* is one plant that almost all of its parts have been used as nutritional supplements and traditional medicines. Moringa leaves contain nutrients, antioxidants, and bioactive substances that have anti-inflammatory, wound healing, and anti-anemia properties.

Purpose: This study aimed to investigate the hematological effect of Moringa leaf powder in male Wistar rats under normal conditions.

Methods: Twenty-four male Wistar rats strain (*Rattus norvegicus*) 9–10 weeks old and 250–275 grams were divided into four groups (n=6), normal as a control group and three other groups were given Moringa leaf powder at doses 200, 400, and 800 mg/kgBW during 12 weeks. Blood samples at week 12 were administered to determine blood count.

Results: The results of this study showed differences between the various doses of Moringa leaf powder for each hematological profile. These differences were more significant for MCH parameters that indicated a decrease in the D800 group compared with the control group.

Conclusion: In conclusion, this study revealed that the consumption of Moringa leaf powder for 12 weeks did not have a significant change in the hematological profile, except for the MCH value that revealed a modification.

Keywords: *Moringa oleifera*, hematological profile, Wistar rats, normal conditions

Introduction

Natural products from medicinal plants and their constituents have been used for the prevention and treatment of various diseases.¹ Nowadays, there is an increase in research focused on traditional medicine: herbs and medicinal plants.² Indonesia is a tropical country with high biodiversity of more than 20,000 plant species, and 35% of them are identified as having health benefits.^{3,4} In Indonesia, consuming herbal plants as traditional medicine is still used to prevent and overcome various health problems.^{5,6} Traditional herbal medicine is most frequently used because it is less expensive, avoids concerns about the side effects of chemical medications, and more closely corresponds with the patient's beliefs.⁷ For example, research on the Meniran plant (*Phyllanthus niruri*) has the potential to function as an immune simulator via blood investigations.⁸ One of the herbal plants in Indonesia that potentially affects blood is Kelor (*Moringa oleifera*).⁶

Moringa oleifera is the most widely cultivated species of a monogeneric family.⁹ This plant can grow in tropical and subtropical regions.¹⁰ In Indonesia, Moringa is known as kelor, also called a Miracle Tree because almost every part of this plant, such as leaves, bark, seeds, flowers, and roots, can be used as nutritional supplements, traditional medicines, and for other industrial purposes.^{11–13} The leaves of *M. oleifera* contain nutrients, antioxidants, chemicals, and bioactive substances.¹⁴ *Moringa oleifera* leaves are an excellent source of iron (28.2 mg/100g), calcium (2003.0 mg/100g), vitamins A (16.3 mg/100g), B-complex, and C as well as minerals.¹⁵ This plant has been recommended by the World Health Organization (WHO) and the United Nations (UN) as an alternative to dietary supplementation in meeting

nutritional requirements.^{16,17} Several studies have been conducted to prove the efficacy of Moringa leaves such as anti-inflammatory, analgesic, antipyretic, antihypertensives, wound healing, anti-diabetic, antiasthma, anticancer, anti-arthritis, anti-epileptic, antiviral, antianemia, and many more.^{14,18–28} Previous study also showed an increased level of hemoglobin, red blood cell count, hematocrit, and total iron content in Sprague Dawley female rat blood after 6 days received Moringa leaves extract.¹⁴ Recent studies of Moringa diet also reported similar results in erythrocyte parameters in Wistar rats.²⁹ Other study stated that Moringa leaves extract can increase the level of hemoglobin, hematocrit, red blood cell, and total white blood cell count on days 8 and 15 of Wistar rats at normal conditions.³⁰

Blood consists of important components that maintain and regulate the body's physiological regulations.³¹ Components of blood consist of plasma and blood cells such as red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).³² Red blood cells play an important function in circulating substances that enter the body, such as oxygen, from the lungs to all the body's cells to carry out metabolic processes.³³ Because of its important function, now blood has become one of the main parameters in preclinical, clinical, and biomedical research in both humans and animals.³⁴ Hematological profiles serve as a tool for evaluating and interpreting health conditions as well as a reference for initial values or controls in a study.³⁵ The presence of disease, metabolic disorders, organ or tissue injury, stress, and the influence of drugs can be seen from changes in hematological profiles.³⁶ For example, high hemoglobin levels and hematocrit values can indicate high feed conversion efficiency.³⁷ According to Takako Fujii's research, resistance training can also improve hemoglobin levels via increasing heme synthesis in the bone marrow.³⁸ For those reasons, authors decided to choose normal conditions rats without induction of chemical substances or exercise for this study.

There is a paucity of data on the hematological effects of Moringa leaf powder in the long term in normal conditions. Knowing the typical physiological values under normal conditions is desirable for the effective application of traditional herbal medicine.³⁷ Thus, this study aimed to investigate the effect of *Moringa oleifera* leaf powder consumption on hematological profile changes (hemoglobin, erythrocytes, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], leukocytes, and thrombocytes) in male Wistar rats under normal conditions.

Materials and Methods

Moringa oleifera Powder and Dose Selection

Moringa leaf powder was obtained from PT. Moringa Organik Indonesia, Blora, Central Java, Indonesia. It has been certified by the Indonesian Food and Drug Authority with a distribution permit BPOM RI MD 619111001777.³⁹

We gave a dose of Moringa leaf powder once to each rat in the intervention group, per oral, once per day at 8 o'clock, for 12 weeks. At the end of this study, the rats were terminated. The blood was collected from cardiac puncture for hematological examination using a Sysmex hematology analyzer (VetScan HM5).

Ethical Statement

The procedures for treatment of the animals were performed according to the guidelines and were approved by the Research Ethics Committee of Universitas Padjadjaran with approval number 910/UN6.KEP/EC/2022.

Animal Model

We obtained 24 male Wistar rats (9–10 weeks old, with body weights within the range from 250 to 275 grams) from PT. Biofarma, Bandung, Indonesia. The rats were acclimatized for 2 weeks and had free access to food and water (*ad libitum*). The dark and light cycle environments were maintained within 12 hours with stable humidity and temperature around ± 22 – 24 °C. They have been acclimated to the animal husbandry conditions of the Animal Physiology Laboratory of Faculty of Medicine, Universitas Padjadjaran (Figure 1).

Experimental Design

The experiments were carried out in 24 male Wistar rats of the *Rattus norvegicus* with body weight 250–275 grams. The rats were acclimatized for 2 weeks under laboratory conditions. Completely randomized design (CRD) is used in this study. The rats were divided into four groups with six animals in each groups ($n=6$). The first group control (K) received standardized food and



Figure 1 Wistar rats after 12 weeks' administration of *Moringa oleifera* powder.

water p.o. for 12 weeks. The second group (D200) received Moringa leaf powder at doses 200 mg/kgBW/day p.o. for 12 weeks. The third group (D400) received Moringa leaf powder at doses 400 mg/kgBW/day p.o. for 12 weeks. The fourth group (D800) received Moringa leaf powder at doses 800 mg/kgBW/day p.o. for 12 weeks. The Moringa leaf powder is dissolved with the same volume of water (2 mL of aquades) for each group.

Assessment Parameters

In this study, blood samples 3 mL were collected at weeks 12 from each rats using 3 cc syringe through left ventricle of heart after inhalation anesthesia with isoflurane solution. The blood samples were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) and analyzed using Sysmex hematology analyzer (VetScan HM5). These blood samples were immediately used for the analysis of hemoglobin (Hb), erythrocytes (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes (WBC), and thrombocytes (PLT).

Statistical Analysis

The data were analyzed by Statistical Package for Social Science (SPSS) software version 26 and were presented as mean with standard deviation (SD). The statistical significance of hematological parameter changes was evaluated by analysis of variance (ANOVA) and associated with post hoc Tukey's test. Differences at p value <0.05 was considered statistically significant.

Results

After 12 weeks, the results were analyzed using the licensed version of SPSS statistics 26. Mean $\pm$ SD was calculated to examine the effect of Moringa leaf powder on the hematological profiles. This study performed normality and homogeneity test on the data. We discovered that all groups' data are normally distributed and homogeneous. In the differences of hematological profiles means, we used the one-way ANOVA test and found significant differences in the MCH value of the D800 group than the control group ($p = 0.04$). Meanwhile, in other hematological profiles, there were differences but not statistically significant (Table 1). Based on further analysis using post hoc Tukey's test to know the differences

Table 1 Hematological Profiles of Male Wistar Rats with Moringa Leaf Powder During 12 Weeks

Hematological Profiles	International Unit	Groups				p value
		K	D200	D400	D800	
Hemoglobin	(g/dL)	14.02 ± 1.04	13.20 ± 0.81	14.5 ± 1.75	13.72 ± 0.92	0.38
Erythrocyte	(10 ¹² /L)	9.00 ± 0.68	8.64 ± 0.69	9.57 ± 1.06	9.35 ± 0.58	0.27
Hematocrit	(%)	51.73 ± 3.39	49.73 ± 2.03	53.46 ± 5.18	49.15 ± 2.29	0.21
MCV	(fL)	55.80 ± 3.27	57.60 ± 3.78	56.17 ± 1.83	52.6 ± 2.41	0.07
MCH	(pg)	15.58 ± 0.54	15.32 ± 0.58	15.15 ± 0.37	14.68 ± 0.33	0.04*
MCHC	(g/dL)	27.12 ± 0.40	26.54 ± 0.94	27.07 ± 1.05	27.92 ± 0.91	0.13
Leukocyte	(10 ⁹ /L)	7.39 ± 2.53	8.24 ± 2.04	9.51 ± 1.40	8.12 ± 3.98	0.60
Thrombocyte	(10 ⁹ /L)	585.00 ± 48.16	540.60 ± 304.29	499.67 ± 109.66	603.80 ± 144.97	0.76

Notes: Values are expressed in means ± standard deviation; *Significant (p<0.05).

Abbreviations: K, control group; D200, Moringa leaf powder at the dose of 200 mg/kg BW; D400, Moringa leaf powder at the dose of 400 mg/kg BW; D800, Moringa leaf powder at the dose of 800 mg/kg BW.

among the groups of MCH value. We found that the mean MCH value of the D800 group was significantly lower than the control group (Table 2).

Hemoglobin is the main component of erythrocytes which is composed of heme (nonprotein) and globin (protein) parts.⁴⁰ Mean of hemoglobin level obtained in the control group was 14.02 g/dL, D200 group was 13.2 g/dL, D400 group was 14.5 g/dL, and D800 was 13.72 g/dL (Table 1). From these results, it can be seen that mean hemoglobin level was slightly higher in the D400 group compared to the other groups. The high levels of hemoglobin in the D400 group were related to the high content of Moringa leaves in protein and amino acids which act as hematopoietic growth factors, as well as vitamin C which plays a role in increasing the absorption of iron into the body.⁴¹ However, the obtained data ranges were not significantly different from the acceptable ranges set above 12 g/dL in rats.^{42,43}

Erythrocytes are hemoglobin-containing blood cells with a biconcave disc shape and no nuclei.⁴³ Mean of erythrocytes level obtained in the control group was $9 \times 10^{12}/L$, D200 group was $8.64 \times 10^{12}/L$, D400 group was $9.57 \times 10^{12}/L$, and D800 was $9.35 \times 10^{12}/L$ (Table 1). From these results, it can be seen that mean erythrocyte level was slightly higher in the D400 group compared to the other groups. The high levels of erythrocytes in the D400 group were related to the content of hematinic agents such as folic acid, vitamin B6, and iron which can stimulate the erythropoietic pathway.⁴⁴

Table 2 Result of Post Hoc Tukey's Test of MCH Value in Different Groups

Groups	Mean Difference	p values
K vs D200	0.26	0.81
K vs D400	0.43	0.44
K vs D800	0.90	0.03*
D200 vs D400	0.17	0.92
D200 vs D800	0.64	0.16
D400 vs D800	0.47	0.36

Note: *Significant (p<0.05).

Abbreviations: K, control group; D200, Moringa leaf powder at the dose of 200 mg/kg BW; D400, Moringa leaf powder at the dose of 400 mg/kg BW; D800, Moringa leaf powder at the dose of 800 mg/kg BW.

However, the obtained data ranges were not significantly different from the acceptable ranges set from $8.32 \times 10^{12}/L$ to $8.88 \times 10^{12}/L$ in male rats.⁴³

Hematocrit is the ratio of erythrocytes to total blood volume expressed as a percentage.⁴⁵ Mean of hematocrit level obtained in the control group was 51.73%, D200 group was 49.73%, D400 group was 53.46%, and D800 was 49.15% (Table 1). From these results, it can be seen that mean hematocrit level was slightly higher in the D400 group compared to the other groups. This result is positively correlated with changes in the number of erythrocytes.⁴⁶ However, the obtained data ranges were not significantly different from the acceptable ranges set from 40% to 50% in rats.^{43,45}

Mean Corpuscular Volume (MCV) is one of the erythrocyte indices used to estimate the average size of erythrocytes.⁴⁷ MCV is obtained by dividing the hematocrit by the number of erythrocytes.⁴⁷ Mean of MCV value obtained in the control group was 55.8 fL, D200 group was 57.6 fL, D400 group was 56.17 fL, and D800 was 52.6 fL (Table 1). From these results, it can be seen that mean MCV value was slightly higher in the D200 group compared to the other groups. However, the obtained data ranges were not significantly different from the acceptable ranges set from 45.9 to 59.9 fL in male rats.⁴³

Mean corpuscular hemoglobin (MCH) is an erythrocyte index that measures the average amount of hemoglobin in red blood cells, expressed in picograms (pg).⁴⁸ This parameter is obtained by calculating the level of hemoglobin divided by the number of erythrocytes per cubic millimeter of blood.⁴⁷ Mean of MCH value obtained in the control group was 15.58 pg, D200 group was 15.32 pg, D400 group was 15.15 pg, and D800 was 14.68 pg (Table 1). From these results, it can be seen that there was a decrease in the mean MCH value for increasing the dose of Moringa leaf powder. However, the value is still within normal limits from 14.6 to 21.3 pg in male rats.^{8,43}

Mean corpuscular hemoglobin is in line with the changes that occur in the level of hemoglobin or the number of erythrocytes. In addition, this parameter also depends on the size of the erythrocytes, because smaller cells cannot contain as much hemoglobin as larger cells.⁴⁹

Mean corpuscular hemoglobin concentration (MCHC) is an erythrocyte index used to determine the erythrocyte saturation index in the blood.⁴³ This examination is obtained by calculating the amount of hemoglobin in 100 erythrocytes and then dividing it by the hematocrit and the results are expressed in grams per deciliter (g/dL).⁴⁷ Mean of MCHC value obtained in the control group was 27.12 g/dL, D200 group was 26.54 g/dL, D400 group was 27.07 g/dL, and D800 was 27.92 g/dL (Table 1). From these results, it can be seen that mean MCHC value was slightly higher in the D800 group compared to the other groups. However, the obtained data ranges were not significantly different from the mean acceptable ranges set at 31.88 ± 1.11 g/dL in male rats.^{8,43}

Leukocytes are cells that function as a defense system or body immunity against pathogens or infectious agent.⁵⁰ Mean of leukocytes level obtained in the control group was $7.39 \times 10^9/L$, D200 group was $8.24 \times 10^9/L$, D400 group was $9.51 \times 10^9/L$, and D800 was $8.11 \times 10^9/L$ (Table 1). From these results, it can be seen that mean leukocyte level was slightly higher in the D400 group compared to the other groups. The high levels of leukocytes in the D400 group were related to the hematopoietic factor of Moringa leaves which has a direct influence on blood production in the bone marrow.^{51,52} However, the obtained data ranges were not significantly different from the mean acceptable ranges set at $7.19 \pm 1.93 \times 10^9/L$ in male rats.⁴³

Thrombocytes are blood cells that play an important role in the process of hemostasis for wound healing.⁵³ Thrombocytes are also produced in the bone marrow and are derived from megakaryocyte whose cytoplasm is fragmented.⁵³ Mean of thrombocytes level obtained in the control group was $585 \times 10^9/L$, D200 group was $540.6 \times 10^9/L$, D400 group was $499.67 \times 10^9/L$, and D800 was $603.8 \times 10^9/L$ (Table 1). From these results, it can be seen that mean thrombocyte level was slightly higher in the D800 group compared to the other groups. However, the obtained data ranges were not significantly different from the mean acceptable ranges set at $1041 \pm 144 \times 10^9/L$ in male rats.⁴³

Discussion

Every part of *Moringa oleifera* can be used as a nutritional supplement and traditional medicine.^{12,13} One of them is the leaves part, which is rich in vitamins, minerals, and effective antioxidants.⁵⁴ Previous studies have proven that *Moringa oleifera* can be used an anti-inflammatory, analgesic, antipyretic, antihypertensive, antidiabetic, accelerating wound healing, anti-cancer, and increasing production of blood cells.^{18–22,24} Despite the numerous studies that have been

conducted on *M. oleifera*, there is paucity of data on the hematological effects of Moringa leaf powder. During its normal condition, it may have good or adverse effects on hematological parameters. Hence, it becomes necessary to investigate hematological profile changes in given *M. oleifera* leaf powder in normal conditions of male Wistar rats.

The hematological profile is useful to assess the health condition and as a reference value of the initial (baseline) or control in a study.^{55,56} The presence of metabolic disorders, diseases, damage to the structure and function organ, the influence of drugs, and stress can be seen from the changes in hematological profile.³⁶ With regard to erythrocyte parameters, the mean values of hemoglobin, erythrocytes number, and hematocrit obtained in rats after administration of Moringa leaf powder were correlated each other and within normal range values. These values are similar to reference data from Marlies de Kort's work.⁴³

Table 1 shows that the rat group given 400 mg/kg BW Moringa leaf powder had higher hemoglobin levels, erythrocyte counts, and hematocrit values than the other groups. This shows that the greater number of erythrocytes in the blood, the greater hematocrit value in the blood.⁴⁶ Hematocrit levels can be decreased by several conditions that cause a decrease production, damage, or the number and size of erythrocytes.⁵⁷ Thus, the hematocrit value is very dependent on the number of erythrocytes because erythrocytes are the cells with the largest mass in the blood.^{46,58} These results are also related to the components of Moringa leaves such as protein amino acids, vitamins B, E, and iron play a role in the synthesis of hemoglobin, the formation and maturation of red blood cells.^{29,41,59}

Table 1 shows a decrease in MCH and MCV values at each increase in the dose of Moringa leaf powder simultaneously. Because, the interpretation of the MCH value must consider the cell volume or MCV value, but is not useful in hypochromic conditions where MCH is not accurately used.⁴⁹ It is possible that MCHC values are different to MCV and MCH values because this parameter is quite sensitive to test errors.⁴⁹

The decrease in MCH values in this study could be influenced by several factors. First, the bioavailability of iron in Moringa leaves is low due to its high phytic acid content.⁶⁰ Phytic acid is the largest storage form of phosphorus in cereals and legumes. Phytic acid is considered an antinutritional compound in foodstuffs because under natural conditions, phytic acid will form bonds with divalent minerals (Ca, Mg, Fe) and proteins to become compounds that are difficult to dissolve.

This causes minerals and proteins to not be absorbed by the body, or their digestibility is low.⁶⁰ Second, iron in Moringa leaves cannot be absorbed properly due to the presence of nonabsorbable complexes with phytic acid, tannins, and some dietary fiber which can bind with iron in the intestinal lumen.⁶¹ In addition, the quercetin content of Moringa leaves can increase iron uptake in the apical enterocyte area but reduce iron output in the basolateral area and reduce the expression of ferroportin (FPN) in the intestine.⁶² In the long term, quercetin and polyphenols can also act as iron chelation if too much.⁶² Third, the environmental temperature can also cause unacceptable shifts in values.^{63,64} MCH, MCV, hematocrit, and platelet parameters stored for less than 4 hours at 33°C exhibits unacceptable bias, so the recommended time limit from collection to processing of blood samples should be adapted to areas where ambient temperatures are high.⁶³ Therefore, the conditioning of rats and the components of Moringa leaf powder need to be further considered comprehensively in future studies.

The highest leukocyte levels appear in the rat group at a dose of 400 mg/kg BW Moringa leaf powder. This result is similar to the study conducted by Ufelle et al, which revealed an increase in total white blood cell count on days 8 and 15 at normal conditions with doses of 150 mg/kg BW and 300 mg/kg BW Moringa leaf powder.³⁰ Another study by Ajugwo et al concludes that Moringa leaves extract has the tendency to increase blood parameters such as white blood cells.⁵¹

According to several studies, rats fed Moringa leaves extract had a slight increase in total leukocyte levels. This can be attributed to the anti-bacterial effects, although the mechanism of action is yet unknown.

The highest thrombocyte levels appear in the rat group at a dose of 800 mg/kg BW Moringa leaf powder. These results are in line with the research study of Kodi et al, which shows that the mean value of total platelets count was increased in rats with a low protein diet added aqueous extract of *Moringa oleifera* for 0, 7, and 14 days.⁶⁵ Similar results were revealed by Amara et al.⁶⁶ The main function of platelets is to maintain hemostasis. *Moringa oleifera* can stimulate platelet proliferation and facilitates hemostasis based on these findings. However, further investigation is necessary to explain this mechanism.

This study had several limitations, including (i) the researchers used the whole compound of Moringa leaf powder so that there is a possibility that it contains anti-nutritional substances; (ii) the researchers did not re-examine the active substance content of the Moringa leaf powder before administering it to the rats; (iii) only one blood sample was taken at the end of the study; and (iv) the hematology profile was the one type of standardized examination used in this study. These limitations will be considered in future studies to improve the reliability of experimental research data.

Conclusion

The results showed that normal rats revealed no increases in hemoglobin, erythrocytes, hematocrit, MCV, MCHC, leukocytes, and thrombocytes cell count on 12 weeks given by Moringa leaf powder. However, Moringa leaf powder at the dose of 800 mg/kgBW/day can significantly decrease the MCH value of normal rats ($p < 0.05$). So, daily administration of Moringa leaf powder (200 mg/kgBW and 400 mg/kgBW) over a period of 12 weeks to normal rats is harmless, and no adverse effects are demonstrated during hematological investigations, but at 800 mg/kgBW doses still need supervision.

However, further research can be conducted on the active substance that has the most effect on the hematological profile, so that only active substance isolates that affect hemoglobin or other parameters. Blood samples were taken that can be done periodically, and a blood smear is used or total iron concentration is calculated to confirm this harmless action.

Acknowledgments

The authors would like to thank to Putri Teesa Radhiyanti Santoso, dr., M.Kes, Ph.D, Hanna, dr., M.Kes, AIFO, Ph.D., Resti Gradia Dwiwina, dr., M.Kes for article reviewer and examiner, and Mr Gilang for assisting in animal treatment. We would also like to thank Universitas Padjadjaran, for funding this research through Riset Disertasi Dosen Universitas Padjadjaran (2203/UN6.3.1/PT.00/2022).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was funded by Universitas Padjadjaran, grant number 2203/UN6.3.1/PT.00/2022 Riset Disertasi Dosen Universitas Padjadjaran.

Disclosure

Mrs Vita Murniati Tarawan reported grants from Universitas Padjadjaran during the conduct of the study. The authors report no other conflicts of interest in this work.

References

1. Kim JH, Kismali G, Gupta SC. Natural products for the prevention and treatment of chronic inflammatory diseases: integrating traditional medicine into modern chronic diseases care. *Evid Based Complement Alternat Med*. 2018;2018:1–2. doi:10.1155/2018/9837863
2. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide research trends on medicinal plants. *Int J Environ Res Public Health*. 2020;17(10):3376. doi:10.3390/ijerph17103376
3. Adnan A, Navia ZI, Silvia M, et al. Diversity of herbs and spices plants and their importance in traditional medicine in the South Aceh district, Indonesia. *Biodiversitas*. 2022;23(7):3836–3843. doi:10.13057/biodiv/d230761
4. Putri YA, Djamel EC, Ilyas R. Identification of medicinal plant leaves using convolutional neural network. *J Phys Conf Ser*. 2021;1845(1):012026. doi:10.1088/1742-6596/1845/1/012026
5. Sinaga RM, Zuska F, Sitorus P. Indegenous healer knowledge about illness and the way to make traditional medicine. *Indonesia J Med Anthropology*. 2021;2(1):43–47. doi:10.32734/ijma.v2i1.5297

6. Parisa N, Kamaluddin MT, Saleh MI, et al. Counseling of the use of herbal medicines for health care to the community. *Indo Com Empowerment J*. 2022;2(1):144–150.
7. El-Dahiyat F, Rashrash M, Abuhamdah S, Abu Farha R, Babar ZUD. Herbal medicines: a cross-sectional study to evaluate the prevalence and predictors of use among Jordanian adults. *J Pharm Policy Pract*. 2020;13(1):2. doi:10.1186/s40545-019-0200-3
8. Fatimah SF, Darmawan E, Narwanti I, Dzulhaifa D, Wulandari IA, Salma RP. Subchronic toxicity test on combination of extracted *Phyllanthus niruri* and *Centella asiatica* on haematology in rats. *J Ilmu Kedokt Kesehat*. 2019;10(3):255–264. doi:10.20885/JKKI.Vol10.Iss3.art8
9. Flora SJS, Pachauri V. *Moringa (Moringa oleifera)* seed extract and the prevention of oxidative stress. In: *Nuts and Seeds in Health and Disease Prevention*. Elsevier; 2011:775–785. doi:10.1016/B978-0-12-375688-6.10092-1
10. Kuete V. *Moringa oleifera*. In: *Medicinal Spices and Vegetables from Africa*. Elsevier; 2017:485–496. doi:10.1016/B978-0-12-809286-6.00022-4
11. Irawan H, Patricio RC, Patricio RC. Indonesian consumers' perceptions of daun kelor (*Moringa oleifera*). *Acta Hortic*. 2017;1158:391–396. doi:10.17660/ActaHortic.2017.1158.44
12. Liu Y, Wang X, Wei X, Gao Z, Han J. Values, properties and utility of different parts of *Moringa oleifera*: an overview. *Chin Herb Med*. 2018;10(4):371–378. doi:10.1016/j.chmed.2018.09.002
13. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: a review on nutritive importance and its medicinal application. *Food Sci Hum Wellness*. 2016;5(2):49–56. doi:10.1016/j.fshw.2016.04.001
14. Mun'im A, Utami Puteri M, Sari SP, A A. Anti-anemia effect of standardized extract of *Moringa oleifera* Lamk. leaves on aniline induced rats. *Pharmacog J*. 2016;8(3):255–258. doi:10.5530/pj.2016.3.14
15. Nurmalasari Y, Rafie R, Warganegara E, Desta Wahyuni L. Pengaruh pemberian ekstrak daun kelor terhadap kadar hemoglobin pada tikus putih galur Wistar jantan. *J Medika Malahayati*. 2021;2021:5.
16. Mahmood KT, Mugal T, Haq IU. *Moringa oleifera*: a natural gift-a review. *J Pharm Sci Res*. 2010;2(11):775.
17. Velázquez-Zavala M, Peón-Escalante IE, Zepeda-Bautista R, Jiménez-Arellanes MA. *Moringa (Moringa oleifera Lam.)*: potential uses in agriculture, industry and medicine. *Rev Chapingo Ser Hortic*. 2016;XXII(2):95–116. doi:10.5154/r.chsh.2015.07.018
18. Bhattacharya A, Naik MR, Agrawal D, Rath K, Kumar S, Mishra SS. Anti-pyretic, anti-inflammatory, and analgesic effects of leaf extract of drumstick tree. *J Young Pharm*. 2014;6(4):20–24. doi:10.5530/jyp.2014.4.4
19. Martínez-González CL, Martínez L, Martínez-Ortiz EJ, et al. *Moringa oleifera*, a species with potential analgesic and anti-inflammatory activities. *Biomed Pharmacother*. 2017;87:482–488. doi:10.1016/j.biopha.2016.12.107
20. Lakshmana Prabu S, Umamaheswari A, Puratchikody A. Phytopharmacological potential of the natural gift *Moringa oleifera* Lam and its therapeutic application: an overview. *Asian Pac J Trop Med*. 2019;12(11):485. doi:10.4103/1995-7645.271288
21. Chin CY, Jalil J, Ng PY, Ng SF. Development and formulation of *Moringa oleifera* standardised leaf extract film dressing for wound healing application. *J Ethnopharmacol*. 2018;212:188–199. doi:10.1016/j.jep.2017.10.016
22. Tshabalala T, Ndhlala AR, Ncube B, Abdelgadir HA, van Staden J. Potential substitution of the root with the leaf in the use of *Moringa oleifera* for antimicrobial, antidiabetic and antioxidant properties. *S Afr J Bot*. 2020;129:106–112. doi:10.1016/j.sajb.2019.01.029
23. Suresh S, Chhipa AS, Gupta M, et al. Phytochemical analysis and pharmacological evaluation of methanolic leaf extract of *Moringa oleifera* Lam. In ovalbumin induced allergic asthma. *S Afr J Bot*. 2020;130:484–493. doi:10.1016/j.sajb.2020.01.046
24. Suphachai C. Antioxidant and anticancer activities of *Moringa oleifera* leaves. *J Med Plant Res*. 2014;8(7):318–325. doi:10.5897/JMPR2013.5353
25. Mahdi HJ, Khan NAK, Asmawi MZ, Mahmud R, Murugaiyah L, Vikneswaran A. In vivo anti-arthritis and anti-nociceptive effects of ethanol extract of *Moringa oleifera* leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. *Integr Med Res*. 2018;7(1):85–94. doi:10.1016/j.imr.2017.11.002
26. Biswas D, Nandy S, Mukherjee A, Pandey DK, Dey A. *Moringa oleifera* Lam. and derived phytochemicals as promising antiviral agents: a review. *S Afr J Bot*. 2020;129:272–282. doi:10.1016/j.sajb.2019.07.049
27. Saleem A, Naureen I. Effect of *Moringa olifera* on haematology and cholesterol level. *Saudi J Biomed Res*. 2021;6(12):298–306. doi:10.36348/sjbr.2021.v06i12.004
28. Kashyap P, Kumar S, Riar CS, et al. Recent advances in drumstick (*Moringa oleifera*) leaves bioactive compounds: composition, health benefits, bioaccessibility, and dietary applications. *Antioxidants*. 2022;11(2):402. doi:10.3390/antiox11020402
29. Coulibaly A, Boua Gngorran N, Baptiste N'guessan Oussou J, Bleyere MN. Evaluation of *Moringa oleifera* Lam leaves (Moringaceae) diets against induced anemia in Wistar rats. *J Anim Physiol Anim Nutr*. 2020;2020:1. doi:10.36349/easjnfs.2020.v02i03.004
30. Onyekwelu K, Ufelle S, Achukwu P, Ezech CO, Ghasi S. Haematological effects of leaf extract of *Moringa oleifera* Lam in normal and myelo-suppressed Wistar rats. *Afr J Biomed Res*. 2018;21:87–90.
31. Molnar C, Gair J. The circulatory system: components of the blood. In: *Concepts of Biology*. 1st ed. BCcampus; 2015.
32. Sharma R, Sharma S. Physiology, Blood Volume; 2022.
33. Pretini V, Koenen MH, Kaestner L, et al. Red blood cells: chasing interactions. *Front Physiol*. 2019;10:10. doi:10.3389/fphys.2019.00945
34. O'Connell KE, Mikkola AM, Stepanek AM, et al. Practical murine hematopathology: a comparative review and implications for research. *Comp Med*. 2015;65(2):96–113.
35. Shrivastav AB, Singh KP. Tigers blood: haematological and biochemical studies. In: *Blood Cell - an Overview of Studies in Hematology*. InTech; 2012. doi:10.5772/50360
36. Ihedioha JI, Ugwuja JI, Noel-Uneke OA, Udeani IJ, Daniel-Igwe G. Reference values for the haematology profile of conventional grade outbred albino mice (*Mus musculus*) in Nsukka, Eastern Nigeria. *Anim Res Int*. 2012;9:1.
37. Onyishi GC, Oguine CC, Nwani SI, Guzie IO, Nwani CD. Haematological parameters dynamics of developing *Gallus gallus domesticus*. *Anim Res Int*. 2017;14(2):2769–2776.
38. Fujii T, Asai T, Matsuo T, Okamura K. Effect of resistance exercise on iron status in moderately iron-deficient rats. *Biol Trace Elem Res*. 2011;144(1–3):983–991. doi:10.1007/s12011-011-9072-3
39. Krisnadi AD. Kelor Super Nutrisi. *Blora*. 2015;2015:1.
40. Chiabrando D, Mercurio S, Tolosano E. Heme and erythropoiesis: more than a structural role. *Haematologica*. 2014;99(6):973–983. doi:10.3324/haematol.2013.091991
41. Yulianti H, Hadju V, Alasiry E. The effect of *Moringa* leaf extract on the hemoglobin levels in young women at SMU Muhammadiyah Kupang. *JST Kesehatan*. 2016;6(3):399–404.

42. Jacob Filho W, Lima CC, Paunksnis MRR, et al. Reference database of hematological parameters for growing and aging rats. *Aging Male*. 2018;21(2):145–148. doi:10.1080/13685538.2017.1350156
43. de Kort M, Weber K, Wimmer B, et al. Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: acceptable value ranges, definition of severity degrees, and vehicle effects. *Toxicol Res Appl*. 2020;4:239784732093148. doi:10.1177/2397847320931484
44. Aghara ID. *A Comparative Study of the Effects of Aqueous Leaf Extracts of Moringa Oleifera and Telfairia Occidentalis on Some Biochemical and Haematological Parameters in Wistar Rats*. University of Nigeria; 2014.
45. Bolliger AP, Everds N. Haematology of the mouse. In: *The Laboratory Mouse*. Elsevier; 2012:331–347. doi:10.1016/B978-0-12-382008-2.00014-3
46. Rosita A, Mushawwir A, Latipudin D. Status hematologis (eritrosit, hematokrit, dan hemoglobin) ayam petelur fase layer pada temperature humidity index yang berbeda. *Student E J*. 2015;4:1.
47. D'Hiru. Live blood analysis setetes darah anda dapat mengungkap status kesehatan dan penyakit yang mengancam anda [Live blood analysis a drop of your blood can reveal the health status and diseases that threaten you]. PT Gramedia Pustaka Umum; 2013. Indonesian
48. Ware AD. The complete blood count and white blood cell differential. In: *Contemporary Practice in Clinical Chemistry*. Elsevier; 2020:429–444. doi:10.1016/B978-0-12-815499-1.00025-9
49. Doig K, Zhang B. A methodical approach to interpreting the red blood cell parameters of the complete blood count. *Am Soc Clin Lab Sci*. 2017;30(3):173–185. doi:10.29074/ascls.30.3.173
50. Carrillo JLM, Rodriguez FPC, Coronado OG, Garcia MAM, Cordero JFC. Physiology and pathology of innate immune response against pathogens. In: *Physiology and Pathology of Immunology*. InTech; 2017. doi:10.5772/intechopen.70556
51. Ajugwo AO, Mounbegna PE, Kemajou TS, Ofokansi VC. Effects of Moringa oleifera leaves extract on haematological parameters of phenylhydrazine anaemia induced Wistar rats. *Int J Public Health*. 2017;2:4.
52. Sembuling K, Sembuling P. *Essential of Medical Physiology*. 6th ed. New Jaypee Brothers Medical Publishers; 2012.
53. Holinstat M. Normal platelet function. *Cancer Metastasis Rev*. 2017;36(2):195–198. doi:10.1007/s10555-017-9677-x
54. Silva MF, Nishi L, Farooqi A, Bergamasco R. The many health benefits of Moringa Oleifera. *J Med Pharm Innov*. 2014;1:3.
55. Bakr S. Hematologic reference values for healthy adult Saudis. *Hematol Transfus Cell Ther*. 2021;43:S53. doi:10.1016/j.htct.2021.10.1065
56. Silitonga M, Silitonga PM. *Haematological Profile of Rats (Rattus Norvegicus) Induced BCG and Provided Leaf Extract of Plectranthus Amboinicus Lour Spreng*. AIP Publishing; 2017:090008. doi:10.1063/1.4995200
57. Dawson WR, Whittow GC. Regulation of body temperature. In: *Sturkie's Avian Physiology*. Elsevier; 2000:343–390. doi:10.1016/B978-012747605-6/50015-8
58. von Borell EH. The biology of stress and its application to livestock housing and transportation assessment. *J Anim Sci*. 2001;79:260–267. doi:10.2527/jas2001.79E-SupplE260x
59. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. *J Med Plant Res*. 2009;3(8):586–591.
60. Gallaher DD, Gallaher CM, Natukunda S, Schoenfuss TC, Mupere E, Cusick SE. Iron bioavailability from Moringa oleifera leaves is very low. *FASEB J*. 2018;31(S1):786. doi:10.1096/fasebj.31.1_supplement.786.13
61. Ndong M, Uehera M, Katsumata S, Sato S, Suzuki K. Preventive effects of Moringa oleifera (Lam) on hyperlipidemia and hepatocyte ultrastructural changes in iron deficient rats. *Biosci Biotechnol Biochem*. 2007;71(8):1826–1833. doi:10.1271/bbb.60644
62. Lesjak M, Hoque R, Balesaria S, et al. Quercetin inhibits intestinal iron absorption and ferroportin transporter expression in vivo and in vitro. *PLoS One*. 2014;9(7):e102900. doi:10.1371/journal.pone.0102900
63. Jain A, Jain S, Singh N, et al. Storage stability of commonly used haematological parameters at 33 °C. *Biochem Med (Zagreb)*. 2018;28(2). doi:10.11613/BM.2018.020901
64. Mazzullo G, Rifici C, Caccamo G, Rizzo M, Piccione G. Effect of different environmental conditions on some haematological parameters in cow. *Ann Animal Sci*. 2014;14(4):947–954. doi:10.2478/aoas-2014-0049
65. Kodi GM, Mustafa HA, Idris AAA. The effects of Moringa oleifera leaves on complete blood count, renal and liver functions as potential therapy for malnutrition. *Mol Cell Biol Sci*. 2022;6(2):55. doi:10.21705/mcbs.v6i2.234
66. Amara AK, Goze BN, Bleyere MN, Yap PA. Moringa oleifera diets effect on haematological parameters of rat (Ratus norvegicus). *J Biosci Med*. 2020;08(05):20–30. doi:10.4236/jbm.2020.85003



Factors Influencing Blood Donation Practice Among Health Care Providers of Public Hospitals in Bahir Dar City, North West Ethiopia: A Case Control Study

Belto Tebabal, Tadele Fentabil Anagaw, Ayinengida Adamu & Desta Debalkie Atnafu

To cite this article: Belto Tebabal, Tadele Fentabil Anagaw, Ayinengida Adamu & Desta Debalkie Atnafu (2023) Factors Influencing Blood Donation Practice Among Health Care Providers of Public Hospitals in Bahir Dar City, North West Ethiopia: A Case Control Study, Journal of Blood Medicine, , 487-498, DOI: [10.2147/JBM.S423013](https://doi.org/10.2147/JBM.S423013)

To link to this article: <https://doi.org/10.2147/JBM.S423013>



© 2023 Tebabal et al.



Published online: 01 Sep 2023.



Submit your article to this journal



Article views: 475



View related articles



View Crossmark data



Citing articles: 4 View citing articles

Factors Influencing Blood Donation Practice Among Health Care Providers of Public Hospitals in Bahir Dar City, North West Ethiopia: A Case Control Study

Belto Tebabal¹, Tadele Fentabil Anagaw², Ayinengida Adamu³, Desta Debalkie Atnafu³

¹Department of Public Health, Amhara Regional Health Bureau, Bahir Dar, Ethiopia; ²Department of Health Promotion and Behavioral Science, School of Public Health, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia; ³Department of Health System Management and Health Economics, School of Public Health, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia

Correspondence: Tadele Fentabil Anagaw, Tel +251-927692916, Email tadele27@gmail.com

Background: Blood donation is a technique in which blood is collected from a healthy individual for transfusion to someone else. WHO estimates that it is necessary to donate blood to 2% to 3% of the country's population to meet blood needs. However, blood donation remains challenging in developing countries.

Objective: This study aimed to identify factors influencing blood donation practices among healthcare providers in public hospitals in Bahir Dar City.

Methods: This institution-based unmatched case-control study was conducted in Bahir Dar City Public Hospitals from May 01 to May 25, 2022. Total sample size was 491 (123 cases and 368 controls) and then the study subject was selected by using simple random sampling technic and collect data through self-administered questionnaire. Bi-variable and multi-variable binary logistic regression analyses were used to determine the association between dependent and independent variables. Finally, the results are presented in charts and tables, and the AOR and CI are reported. Statistical significance was set at $P < 0.05$.

Results: Fear of anemia (adjusted odds ratio (AOR): 0.02; 95% CI 0.007–0.078), lack of opportunity (AOR: 0.42; 95% CI 0.22–0.83), lack of time (AOR: 0.03; 95% CI, 0.005–0.199), profession (AOR: 0.15; 95% CI, 0.05–0.42), aware of free medical checkup (AOR: 31.79; 95% CI 13.13–76.94), willingness to donate blood (AOR: 5.09; 95% CI 2.25–11.50), blood group type (AOR: 5.67; 95% CI 1.42–22.68), and higher work experience (AOR: 7.99; 95% CI 2.59–24.67) were found to be significantly associated with blood donation practice.

Conclusion: This study revealed that multiple factors influenced the practice of blood donation among healthcare providers. Therefore, access to blood donation areas and emphasizing the importance of donor blood donation are important for facilitating blood donation.

Keywords: blood donation, blood donation practice, case control, health care providers

Introduction

Blood donation is the practice of gathering blood from willing donors, who are unlikely to become infected or whose health is negatively affected by blood.¹ Blood donation is a technique in which blood is collected from a willing and healthy person for the purpose of transfusion to someone else.²

The availability of blood varies significantly between low- and high-income nations. A country's general blood supply can be determined by examining the percentage of whole-blood donations. In high-income nations, the median blood donation rate is 31.5 donations per 1000 individuals. As a comparison, 16.4 donations per 1000 people were made in upper-middle-income countries, 6.6 donations per 1000 people were made in lower-middle-income countries, and 5.0 donations per 1000 people were made in low-income countries.³

Studies in different area show there is low practice of blood donation among health care workers. In Nigeria Benin Teaching Hospital medical practitioners 41.4%, Ethiopia Arsi Negele Hospital 39.5%, Tigray region hospital 47.8% and Tikur Anbessa Hospital 38.3% and University of Gondar Hospital 33.2%.⁴⁻⁸

Knowledge, attitude, sex, age, and presence of family members or relatives who have received blood among medical personnel, profession, higher work experience, married health professionals, adequate education, inaccessibility blood bank, fear of anemia, and not being asked to donate blood are factors associated with blood donation practice.⁴⁻⁶

Millions of people require blood donations during various medical emergencies and circumstances. The various medical conditions that require blood to remain alive are insufficient to ensure a safe blood supply to people in need in Ethiopia.⁷

WHO estimates that it is necessary to donate blood to 2% and 3% of the country's population; to meet the most basic blood needs of a country, but in Ethiopia less than 0.5% of the population of donate blood.⁸ Blood transfusions save lives and improve health, but many patients needing blood transfusions do not have timely access to a safe blood source.⁹

Study shows that the gap between the demand and supply of blood is wide in many low- and middle-income countries, with a global annual blood demand of 304,711,244 and supply of 272,270,243 blood product units for a demand/supply ratio of 1.12. Out Of 195 countries, 119 countries (61%) did not have an adequate blood supply to meet their needs. In these 119 countries, the unmet need totals 102,359,632 units of blood products or 1849 units per 100,000 people worldwide. Not all countries in Central, Eastern, and Western sub-Saharan Africa, Oceania, and South Asia have enough blood to meet their needs.¹⁰ Most of the countries in the world are facing a concerning shortage of blood due to a decrease in donations.^{11,12} This is the worst blood shortage in more than 10 years and poses the risk of raising concerns about patient care.¹²

Barriers to blood donation include medical reasons, fear (needles, dizziness, etc.), lifestyle barriers, lack of marketing communication, lack of knowledge about blood donation, and negative experiences related to blood donation.¹³ Rapid access to blood transfusions plays an important role in reducing the morbidity and mortality rates in healthcare settings. Although the global demand for blood has gradually increased, there is still evidence of blood shortages and blood products in developing countries, including Ethiopia.¹⁴

According to study done at Addis Ababa, blood availability is insufficient to meet the increased demand for blood and its components. The National Blood Bank of Ethiopia collects nearly 200,000 units of donor blood each year, which is equivalent to 200,000 pints/94,000 liters. The country requires 18,000 units of blood (18,000 pints/8460 liters) per day, but the average daily blood collection is approximately 1100 units (1100 pints/517 liters), which is a shortfall of 16,900 pints/7943 liters.¹⁵

Pregnant women, cancer patients, and individuals with an illness that causes anemia often have very important transfusion needs, which complicate a blood crisis because these patients require components or specific blood products.^{16,17} Patients who have experienced serious injuries from car crashes,¹⁸ when the demand for blood is too high, anemia leads to significant morbidity and mortality.⁷

Continual work is required to create new frameworks and methods that will aid in enlisting more blood donors, owing to the imbalance between the supply and demand for blood.^{19,20} In Ethiopia, considerable research has been conducted on blood donation at a community level. However, little research has been conducted on the practice of blood donation among health care providers.

Sub-Saharan Africa (SSA) has a very high maternal mortality rate (MMR) according to a global assessment for the year 2019, with a point estimate for 2017 of 542 (UI 498–649) maternal deaths per 100,000 live births. Up to 72% of maternal deaths are attributable to the inability to treat severe fetal hemorrhage with blood. Delayed transfusion has also been linked to higher newborn mortality.²¹ This study aimed to identify the factors influencing blood donation practices among the healthcare providers of public hospitals in Bahir Dar City, North West Ethiopia.

Methods and Materials

Study Design and Period

An unmatched facility-based case-control study was conducted at public hospitals from May 1 to May 25, 2022, in Bahir Dar City, Ethiopia.

Study Area and Setting

The study was conducted at Bahir Dar City Public Hospital, the capital of the Amhara regional state of northwest Ethiopia, 560 km from Addis Ababa. The region has an elevation of 1801 m above sea level. Total population of Bahir Dar city were 345 084.²² Bahir Dar City administration has 28 governmental health facilities, including three public hospitals, Tibebe Ghion specialized hospital (TGSH), Felege Hiwot comprehensive specialized hospital (FHCSH) and Addis Alem primary hospitals (AAH). Forty-three (43) blood banks are available all over the country in Ethiopia, including ten blood bank centers in Amhara region. Ten blood bank sites serve for blood collection, distribution to hospitals and share information on blood donation to increase blood donation in Amhara region and save people's life by delivering blood. Bahir Dar city public hospitals have blood dispensing units that are collected by the blood banks.

Sources Populations

All health care providers work in public hospitals in Bahir Dar city.

Study Populations

For Cases: Healthcare providers with blood donation practice during or within three years of the study period.

For Controls: Healthcare providers did not have blood donation practices during or within three years of the study period.

Inclusion Criteria

For cases, healthcare providers with blood donation practices in the RBB before or during the study period.

Controls: Health care providers without blood donation practice before or during the study period.

Exclusion Criteria

Cases: Healthcare providers with blood donation practices outside the Bahir Dar district, donated beyond the last three years, with sick leave, annual leave, and maternity leave were excluded from the study.

Controls: Healthcare providers without blood donation practices with sick leave, annual leave, and maternity leave were excluded from the study.

Study Variables

Dependent variables: Blood donation practice.

Independent variables: Sociodemographic characteristics: Age, Sex, Religion, Marital status, educational status, Profession, Work experience, easy accessibility of the blood bank, Attitude and Knowledge of blood donation practice.

Operational Definition

Cases: Healthcare providers with blood donation practice at RBB during and before three years of the study period.

Controls: Health care providers without blood donation practice during and before the study period.

Blood donation practice, having at least one history of blood donation within the last three years in blood bank, was used to label them as having practice.²³

Knowledge on blood donation: was measured by using seven questions "of them two questions values five and six points each" to assess knowledge of blood donation. For the "correct" and "incorrect" response, "1" and "zero" scores were used, respectively. The total score was obtained by summing the scores for the seven knowledge questions. The scoring ranged from 0 to 16. Those participants who were answered "eight" or more questions correctly from 16 ($\geq 50\%$) were considered as knowledgeable.²⁴

Attitude: was measured using 11 questions and responses were coded as if correctly answered as 1 and if not correctly answered 0. The total attitude score was calculated by summing up the individual's response that range from 0 to 11. Study participants who scored $>+50\%$ had favorable attitude and below half were unfavorable attitude. Attitudes: In this study, attitudes were assessed using 11 questions. Similar to knowledge scoring "1" and "zero" were used for favorable

and unfavorable attitude, respectively. A total score was calculated to determine the total attitude score. The scores ranged from 0 to 11. Attitude scores of half and more were considered favorable.²⁴

Never donors: if participants do not donate blood in their lives.

Ever donated: Respondents who donate once or more in their lifetime.²⁵

Sample Size Determination and Sampling Procedures

The sample size was calculated based on the double population proportion formula using Epi Info software version 7.2.5.0, and the following assumptions were made: 95% confidence, 90% power, 1:3 ratio of case -to- control, and 10% non-response rate. Based on these assumptions, the reasonable sample size was calculated as 491 (123 cases and 368 controls)²⁶ (Table 1).

Data was collected from the three public hospitals, namely TGSH, FHCSH and AAH in Bahir Dar city, with a total of 1766 health care providers. Three public hospitals' health care workers were included in the study with the total number of health care providers in each hospital were at TGSH (707), FHCSH (903) and AAH (156). After identifying cases and controls that means who had blood donation practices and non-donors at each hospital proportional allocation of cases and controls were done. The cases and controls of each hospital were TGSH (49,147), FHCSH (63,189) and AAH^{11,28} respectively. Finally, cases and controls were selected by using simple random sampling technique by using random number table in each selected facility.

Data Collection Tools and Techniques

Data collection tool was adapted from similar literatures.^{26,29,30} A structured self-administered questionnaire was used to collect data from the participants. Six trained nurses were recruited as data collectors, and the principal investigator, as a supervisor, followed the data collection activities. The data collectors received one day training during the data collection procedures. Prior to the primary data collection process, the questionnaire was pretested with 25 healthcare professionals (5% of the sample health care providers) at the neighboring Merawi Hospital. After the pretest, a few questions in the questionnaire were revised for clarity, logical order, skip patterns, and wordiness.

Data Quality Assurance

The questionnaires were translated into the Amharic language and then back into English for consistency. A day of training was provided to the data collectors. The principal investigator controlled the overall activity. Data quality was controlled by pretest the questionnaire before the actual data collection for 5% of the sample size and modifying to the proper data collection materials after pilot study. All the completed questionnaires were examined for completeness and consistency during data management, storage, and analysis.

Data Management and Analysis

The collected data were entered using Epi-data version 3.1 statistical software and then exported to SPSS version 23 for analysis. Descriptive statistics of the collected data were performed using relevant statistical parameters such as frequency and percentage. At 25% level of significance bi-variable binary logistic regression analysis was performed to screen for potentially significant independent variables and using significant independent variables multivariable binary logistic regression analysis was performed to determine the association between the dependent variable and independent variables. The Hosmer-Lemeshow goodness-of-fit test was used to check the adequacy of the final model,

Table 1 Sample Size Calculation of Influencing Factors of Blood Donation Practice in the Previous Study Conducted in North East Ethiopia, 2021²⁷

Variables	AOR	% of Cases Exposed	% of Controls Exposed	Sample Size
Age ≥25	2.3	55.4%	35.1%	354
Favorable attitude	2.41	81.4%	64.54%	423
Knowledge	2.09	54.9%	36.84%	446

and the model was fitted to the data. For multivariable (covariates) binary logistic regression, 95% confidence interval was computed, and variables with p-value <0.05, were considered as statistically significant for the dependent variable.

Results

Socio Demographic Characteristics

This study had a response rate of 100% with 123 cases and 368 controls a total of 491 participants, from the total participants 226 (61.41%) of controls and 42 (34.15%) of cases were in the age group of 23–29 years; 210 (57.1%) of controls and 89 (72.4%) of cases were male healthcare providers; 332 (90.22%) of controls and 112 (91%) of cases were orthodox; 215 (58.4%) of controls and 84 (68.3%) cases were married; 293 (79.62%) of controls and 65 (52.84) of cases were Bachelors (BSc) in educational status; 194 (52.72%) of controls and 42 (34.15%) of cases were nurse professionals; 229 (62.23%) of controls and 54 (43.9%) of cases were 0–6 years' work experience (Table 2).

Easily Accessibility of Blood Bank Donation Center

From a total of 491 participants, 123 cases and 368 controls, 265 (72.01%) of controls, and 95 (77.2%) of cases of health care providers had blood bank access.

Knowledge Related Factors

Of the total participants, 225 (61.14%) controls and 73 (59.35%) cases were responded good health as criteria for donating blood; minimum interval of blood donation was 263 (71.5%) controls and 104 (84.55%) cases were accurate responses; time required for donation was 229 (62.22%) controls and 87 (70.73%) cases had inaccurate responses;

Table 2 Socio Demographic Characteristics of Health Care Providers Who are Working in Public Hospitals, Bahir Dar City, Ethiopia, July 2022. (N = 491)

Variables	Category	Control %	Cases %	X ²		
				P-x ²	df	P-value
Age in years	≤22yrs old	4.07	3.25	30.23	2	0.000
	23–29yrs old	61.41	34.15			
	≥30yrs old	34.51	62.60			
Gender	Female	42.93	27.6	9.05	1	0.003
	Male	57.1	72.4			
Religion	Orthodox	90.22	91.0	14.51	3	0.002
	Muslim	5.4	6.5			
	Protestant	4.35	0			
	Others	0	2.44			
Marital Status	Single	37.77	21.9	15.15	3	0.002
	Divorced	2.72	8.13			
	Widowed	1.1	1.6			
	Married	58.4	68.3			
Educational status	Diploma	6.8	6.5	47.19	3	0.000
	Bachelors (BSc)	79.62	52.84			
	Masters	4.1	5.7			
Professions	Medical doctor	9.5	34.96	52.06	4	0.000
	Physician	9.5	34.96			
	Nurse	52.72	34.15			
	Laboratory	7.1	12.2			
	Midwifery	9.5	4.88			
	Others (Anesthesia, radiology)	21.2	13.82			
Work experience	0–6yrs	62.23	43.9	14.16	2	0.001
	7–12yrs	27.99	37.4			
	≥13 yrs.	9.82	18.7			

benefits of blood donation 265 (72.01%) controls and 90 (73.17%) cases were responded happiness; 167 (45.38%) of controls 68 (55.28%) cases had adequate knowledge (Table 3).

Attitudinal Influencing Factors

Of the total participants, 207 (56.25%) controls and 96 (78.05%) cases were family members or close friends who ever needed blood transfusion, 187 (50.81%) of controls and 81 (65.85%) of cases had family members or close friends who donated blood. Two hundred and eighty-eight (78.26%) controls and 108 (87.80) patients were overall favorable attitude (Table 4).

Table 3 Knowledge-Related Influencing Factors of Blood Donation Practice Among Health Care Providers of Public Hospitals, Bahir Dar City, Ethiopia, July 2022 (n = 491)

Variables	Category	Control %	Case %	x ²		
				Pearsons x ²	df	P value
Criteria for donating blood	Being voluntary					
	No	30.7	27.64	0.41	1	0.521
	Yes	69.3	72.36			
	Age 18–65 yrs					
	No	45.4	41.46	0.57	1	0.449
	Yes	54.62	58.54			
	Weight >45kg					
	No	41.66	35.77	1.29	1	0.256
	Yes	58.42	64.23			
	Good health					
Minimum interval can a person donate blood	No	38.86	40.65	0.12	1	0.725
	Yes	61.14	59.35			
	Inaccurate respond	28.53	15.45	8.36	1	0.004
	Every 3 month	71.5	84.55			
	Inaccurate respond	29.89	30.89	0.04	1	0.834
	<500mL	70.1	69.1			
	Inaccurate respond	65.22	59.35	1.37	1	0.241
	24–48hrs	34.78	40.65			
	Inaccurate respond	62.22	70.73	2.91	1	0.088
	<20min	37.77	29.27			
Health benefits of blood donation	Free medical checkup					
	No	64.13	7.32	119.02	1	0.000
	Yes	35.87	93.68			
	Happiness					
	No	27.98	26.82	0.62	1	0.803
	Yes	72.01	73.17			
	Control weight					
	No	57.06	60.2	0.36	1	0.547
	Yes	42.93	39.84			
	Lower cancer risk					
Can blood be artificially manufactured?	No	62.23	53.67	2.82	1	0.093
	Yes	37.77	46.34			
	Reduce heart attack					
	No	58.97	62.60	0.51	1	0.477
	Yes	41.03	37.4			
	Yes	13.86	21.14	3.69	1	0.055
	No	86.14	78.86			
	Inadequate	54.61	44.71	3.62	1	0.057
	Adequate	45.38	55.28			
	Overall, Knowledge					

Table 4 Attitudinal Influencing Factors of Blood Donation Practice Among Health Care Providers of Public Hospitals, Bahir Dar City, Ethiopia, July 2022 (N = 419)

Variables	Category	Controls (%)	Cases (%)	χ^2		
				Pearson's	Df	p-value
What do you think about blood donation?	Good	89.94	95.93	4.23	1	0.040
	Bad	10.05	4.1			
What do you think is the best source of blood donation?	Voluntary	75.3	69.92	1.37	1	0.242
	I do not know	24.73	30.08			
Any family member or close friend who ever needed blood transfusion?	Yes	56.25	78.05	18.54	1	0.000
	No	43.75	21.95			
Any family member or close friend who donates blood?	Yes	50.8	65.85	8.41	1	0.004
	No	49.2	34.15			
Is blood donation harmful to donor?	Yes	13.31	9.76	1.07	1	0.3
	No	86.7	90.24			
Should patient relative be asked to donate?	Yes	50.81	55.3	0.74	1	0.390
	No	49.2	44.7			
Do you encourage relatives to donate?	Yes	69.56	73.2	0.57	1	0.448
	No	30.43	26.83			
Have you donated blood	Yes	0	100			
	No	100	0			
What motivates you to donate blood	Aware of the lack of blood supply		58.54			
	Health benefits		45.53			
	Existence of an involved blood donor		13.82			
	Family members		14.63			
Where you was donate blood	Regional blood bank Bahir Dar district		100			
Reasons for not donated blood?	Lack of info					
	Yes	13.31	8.13	2.34	1	0.126
	No	86.7	91.87			
	Lack of opportunity					
	Yes	64.94	47.15	12.21	1	0.000
	No	35.05	52.84			
	Lack of time					
	Yes	11.41	1.63	10.82	1	0.001
	No	88.6	98.3			
	Fear of Anemia					
	Yes	41.85	4.06	60.10	1	0.000
	No	58.15	95.93			
	Religious					
	Yes	5.7	1.63	3.44	1	0.064
	No	94.3	98.3			
Blood group type?	Do not know	14.67	3.25	19.03	4	0.001
	A	24.45	20.32			
	B	20.65	43.95			
	AB	10.87	13			
	O	29.35	28.45			
Willingness to donate blood in the future	No	32.33	17.07	12.09	1	0.001
	Yes	67.66	82.93			
Overall Attitude	Unfavorable	21.73	12.19	5.38	1	0.020
	Favorable	78.26	87.80			

Reasons for Not Practicing Regularly Donate Blood

Of the total participants, the reason not to practice/regularly donate blood were 239 (64.94%) of controls 58 (47.15%) of cases were responded lack of opportunity; 154 (41.85%) of controls 5 (4.06%) of cases feared of anemia respectively; mainly identified reasons that not to donate/not to donate blood regularly (Figure 1).

Factors Associated with Blood Donation Practice

The variables age, sex, marital status, education, work experience, profession, how often an individual donates blood, awareness of free medical checkup, blood donation, family member, or close friend who ever received blood transfusion, family member or close friend who donated blood, lack of opportunity, fear of anemia, lack of time, blood group, willingness to donate blood, and overall attitude were associated with the practice of blood donation on Bivariable binary logistic regression at a P-value of less than or equal to 0.25. The results of the Bivariable logistic regression were entered into a multivariable binary logistic regression analysis.

Thus, fear of anemia, lack of opportunity, lack of time, medical doctor by profession, awareness of free medical checkups, willingness to donate blood, blood group type, etc. Work experience was significantly associated with blood donation practice.

The odds of blood donation practice among healthcare providers who reported fear of anemia were 98% lower than the odds for health care providers free from fear of anemia (AOR 0.02 95% CI 0.007–0.078). The odds of blood donation practices by healthcare providers who reported lack of opportunity were 58% lower than the odds for health care providers that they had an opportunity (AOR: 0.42 95% CI 0.22–0.83). The odds of blood donation practice among healthcare providers who reported lack of time were 97% lower than the odds for health care providers free from (AOR: 0.03 95% CI 0.005–0.199). The odds of blood donation practice were 31.79 times higher aware of free medical checkup among non-aware of free medical checkup (AOR: 31.79 95% CI 13.13–76.94). The odds of blood donation practice of health care providers were 5.09 times higher willingness to donate blood than among non-willingness to donate blood (AOR: 5.09 95% CI 2.25–11.50). The odds of blood donation practice in the common blood group were 5.67 times higher than those among those who did not know their blood group status (AOR: 0.5.67 95% CI 1.42–22.68). The odds of blood donation practice of health care providers were 7.99 times higher blood donation practice of work experience of ≥ 13 years than among 0–6 years' (AOR 7.99 95% CI 2.59–24.6) (Table 5).

Discussion

In keeping with the objective of this study, an effort was made to identify the variables influencing the practice of blood donation among healthcare providers in public hospitals. The study found that the following variables influenced the practice of blood donation: fear of anemia, lack of opportunity, lack of time, MD by profession, awareness of free medical checkups, willingness to donate blood, blood group type, and work experience.

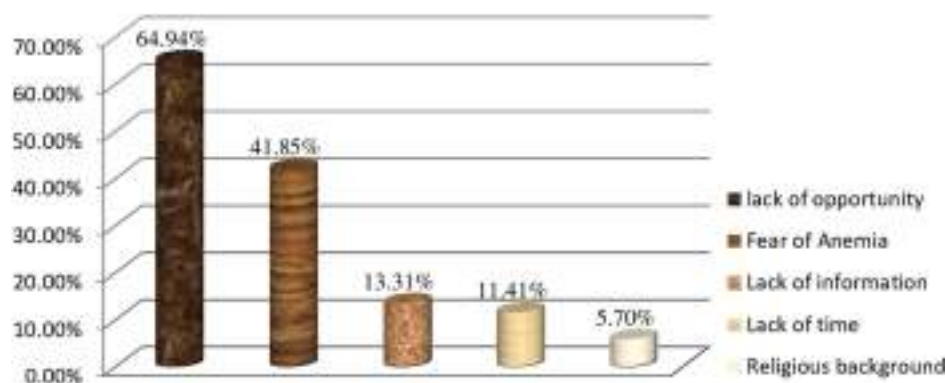


Figure 1 Reasons for not practicing blood donation among healthcare providers at public hospitals, 2022, Bahir Dar city, Ethiopia.

Table 5 Multiple Logistic Regression Analysis of Factors of Blood Donation Practice Among Health Care Providers of Public Hospitals, Bahir Dar City, Ethiopia, July 2022 (N = 419)

Variables		Cross Tabs		COR with 95% CI	AOR with 95% CI	P-value
		Case	Control			
Work experience	0–6yrs	54	229		1	0.000
	7–12yrs	46	103	1.89 (1.19–2.99)	2.57 (1.24–5.37)*	
	>13yrs	23	36	2.71 (1.48–4.94)	7.99 (2.59–24.67)*	
Profession	Physician	43	35		1	0.000
	Nurse	42	194	0.17 (0.10–0.30)	0.23 (0.09–0.59)*	
	Laboratory	15	26	0.47 (0.22–1.02)	0.47 (0.12–1.90)	
	Midwife	6	35	0.14 (0.05–0.37)	0.09 (0.03–0.35)*	
	Others	17	78	0.17 (0.08–0.35)	0.15 (0.05–0.42)*	
Free medical checkup awareness	prof					0.000
	No	9	236		1	
	Yes	114	132	22.65 (11.12–46.12)	31.79 (13.13–76.94)*	
Lack of opportunity is the reason not to donate blood	No	65	129		1	0.013
	Yes	58	239	0.48 (0.32–0.73)	0.42 (0.22–0.83)*	
Fear of anemia is the reason not to donate blood	No	118	214		1	0.000
	Yes	5	154	0.06 (0.02–0.15)	0.02 (0.007–0.078)*	
Lack of time is the reason not to donate blood	No	121	326		1	0.000
	Yes	2	42	0.13 (0.03–0.54)	0.03 (0.005–0.199)*	
Know Blood group type	No	4	54		1	0.014
	A	25	90	3.75 (1.23–11.36)	13.48 (3.15–57.69)*	
	B	43	76	7.63 (2.58–22.54)	11.32 (2.80–45.59)*	
	AB	16	40	5.40 (1.67–17.38)	6.77 (1.43–32.13)*	
	O	35	108	4.37 (1.47–12.94)	5.67 (1.42–22.68)*	
Willingness to donate	No	20	120		1	0.000
	Yes	103	248	2.49 (1.47–4.22)	5.09 (2.25–11.50)*	

Note: *Shows significant variable associated with outcome variable.

Health care providers who fear anemia were 98% less likely practicing blood donation than healthcare providers among free from fear of anemia; this result is slightly higher than studies conducted in ALERT Hospital, Ethiopia³⁰ Fear of anemia and not approaching donation were the leading reasons for non-donors. The fact that our study only considered healthcare providers from public hospitals, where participants might have higher exposure, a reliable source of information, and access to blood bank donation centers, may be one of the most plausible causes of the disparities.

Health care providers who thought there was lack of opportunities were 58% less likely to practice blood donation compared to health care providers who thought there were opportunities. This outcome was in line with study conducted in Nigeria²⁷, which may indicate that lack of opportunity is one of influencing factors for blood donation practice. This can be accomplished by fostering opportunities, particularly among healthcare workers, and the need for action to be taken, which gives individuals a chance to expose healthcare providers to practice blood donation.

The odds of voluntary blood donation practice in health care providers who reported lack of time were 97% less likely to practice blood donation compared to health care providers who thought that they had time to donate. This outcome is consistent with that of studies conducted in Nigeria and Ethiopia.^{27,29} This might be due to the high workload of the hospitals. Therefore, responsible bodies will establish a connection with healthcare institutions, inform them of onsite blood collection time, and make regular use of mobile blood supply facilities.

Health professionals of nursing and Midwife health care providers were 77% and 91% less likely to practice blood donation compared to MDs by profession, respectively. This study is in line with study in Tigray Health healthcare providers with a degree at their level of education were 69% less likely to donate blood than specialists.³¹ This might be the difference in level of understanding. The higher the status of health professional knowledge, the more practicing

blood donation this study is in line with studies done in Bahir Dar Health professionals who were more likely than other professions to donate blood voluntarily.³²

Healthcare providers who were aware of free medical checkup were 31.79 times higher blood donation practice than those who were unaware of free medical checkups. Among people who had never donated blood, the blocking factor was fear of the announcement of test results in 6.57%, and fear of health problems after screening (39.3%) was the main reason cited by non-donors.

The odds of practicing blood donation among healthcare providers who were willing to practice blood donation were 5.09 times higher compared to those among healthcare providers with non-willingness. This outcome is consistent with research conducted in various parts of Ethiopia. The results were slightly higher than those of studies conducted in Chiro town of Eastern Ethiopia and lower than those of studies conducted among students at Madda Walabu University and Bale Robe town in Southeast Ethiopia.^{28,33,34} This discrepancy might be due to differences in the study population and sociodemographic factors, suggesting that willingness to donate blood is a crucial variable that significantly influences practices related to blood donation.

The odds of practicing blood donation among healthcare providers of common blood groups were 5.67 times higher than those of healthcare providers who did not know their blood group type. All common and ever-rare blood groups were represented by participants in their study. This study implies that healthcare professionals donate blood voluntarily and that they do so when there is a need, particularly when there is a scarcity of blood from common blood groups. This study contrasts with that conducted in Northern India.³⁵ This is because of the differences in the study populations and the reasons why they were aware of their own blood groups.

Healthcare providers with >13 years of work experience were 7.99 times higher blood donation practice than compared with health care providers and 0–6yrs. This outcome was similar to the study being practical in Tigray regional state public hospitals, northern Ethiopia and Arsi. In comparison to those with >11 years of work experience, those with one to five years of work experience were less likely to donate blood.^{5,23} This might be because those with more experience were more frequently exposed to blood donation than those with less experience.

Conclusion

This study revealed that multiple factors influenced the practice of blood donation among healthcare providers. Type of profession, fear of anemia, access to blood bank, lack of time, and work experience were determinant factors for blood donation. Therefore, access to blood donation areas and emphasizing the importance of blood donation for both donors and patients who need blood are important for facilitating blood donation.

Data Sharing Statement

All the necessary data are available from the corresponding author and will be provided upon reasonable request.

Ethics Approval and Consent to Participate

Ethical clearance was obtained from the institutional review board of Bahir Dar University, College of Medicine and Health Sciences with ethical review board (IRB). Written informed consent was obtained from all the participants. The study participants provided written informed consent to withdraw at any point in the data collection, and the data were confidentially secured. All methods were carried out in accordance with the relevant guidelines and regulations and complied with the Helsinki Declaration.

Acknowledgments

This article is presented as part of thesis at Bahir Dar university “Factors Influencing Blood Donation Practice Among Health Care Providers Of Public Hospitals In Bahir Dar City, North West Ethiopia, A Case Control Study” <https://ir.bdu.edu.et/handle/123456789/15220>. We would like to thank Bahir Dar University, data collectors, supervisors, and the study participants.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

All authors declare that they have no competing interests for this work.

References

- World Health Organization. *Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation*. World Health Organization; 2012.
- Nureye D, Tekalign E. Opportunities and challenges of blood donation and blood therapy in Ethiopia. *Health Sci*. 2019;8:122–127.
- World Health Organization. *Global Status Report on Blood Safety and Availability 2021*. World Health Organization; 2022.
- Urgesa K, Hassen N, Seyoum A. Knowledge, attitude, and practice regarding voluntary blood donation among adult residents of Harar town, Eastern Ethiopia: a community-based study. *J Blood Med*. 2017;8:13. doi:10.2147/JBM.S121460
- Yirgu AN, Mohammed KH, Diriba SD, Babso AK, Abdo AA. Blood donation and associated factors among employees working at Negele Arsi General Hospital and Medical College, Southeast Ethiopia: a cross-sectional study. *J Blood Med*. 2021;12:475. doi:10.2147/JBM.S301826
- Arage G, Ibrahim S, Adimasu E. Blood donation practice and its associated factors among health professionals of University of Gondar Hospital, Northwest Ethiopia: a cross sectional study. *BMC Res Notes*. 2017;10(1):1–6. doi:10.1186/s13104-017-2618-5
- Getie A, Wondmienieh A, Bimerew M, Gedefaw G, Demis A, Onida F. Blood donation practice and associated factors in Ethiopia: a systematic review and meta-analysis. *Biomed Res Int*. 2020;2020:1–8. doi:10.1155/2020/8852342
- World Health Organization. *Screening Donated Blood for Transfusion-Transmissible Infections: Recommendations*. World Health Organization; 2010.
- World Health Organization. *Blood Safety and Availability*. World Health Organization; 2020.
- Roberts N, James S, Delaney M, Fitzmaurice C. The global need and availability of blood products: a modelling study. *Lancet Haematol*. 2019;6(12):e606–e615. doi:10.1016/S2352-3026(19)30200-5
- Blood Shortage Canadian C. Drought in Namibia the Namibian; 2013.
- Warner MA, Patel PA, Hensley NB, Mazzeffi M. COVID-19-related blood shortages and cardiac surgery: do we have too many eggs in one basket? *J Cardiothorac Vasc Anesth*. 2022;36(7):1823–1826. doi:10.1053/j.jvca.2022.02.021
- Abdelgader A, Al Ghumlas AK. The future of voluntary blood donation in the Kingdom of Saudi Arabia. *Transfusion*. 2020;60(S1):S28–S34. doi:10.1111/trf.15683
- Mohammed S, Essel HB. Motivational factors for blood donation, potential barriers, and knowledge about blood donation in first-time and repeat blood donors. *BMC Hematol*. 2018;18(1):1–9. doi:10.1186/s12878-018-0130-3
- Gurmu ED, Gezaew MA, Asfaw YM. Utilization of blood and blood products among pediatric patients admitted to St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. *Ethiopian J Pediatr Child Health*. 2021;16(2):1–13.
- Guan W-J, Z-y N, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708–1720. doi:10.1056/NEJMoa2002032
- Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. *Ann Intern Med*. 2020;173(5):362–367. doi:10.7326/M20-3012
- Allon R, Epstein D, Shavit I. Prehospital transfusion of low titer cold-stored whole blood through the intraosseous route in a trauma patient with hemorrhagic shock. *Transfusion*. 2020;60(4):875–878. doi:10.1111/trf.15732
- Li L, Li KY, Yan K, et al. The history and challenges of blood donor screening in China. *Transfus Med Rev*. 2017;31(2):89–93. doi:10.1016/j.tmr.2016.11.001
- Bagot KL, Murray AL, Masser BM. How can we improve retention of the first-time donor? A systematic review of the current evidence. *Transfus Med Rev*. 2016;30(2):81–91. doi:10.1016/j.tmr.2016.02.002
- World Health Organization. *WHO African Region Status Report on Blood Availability, Safety and Quality*. World Health Organization; 2022.
- Ambaye E, Regasa ZW, Hailiye G. Early initiation of antenatal care and its associated factors among pregnant women attending antenatal care at public health centres in Bahir Dar Zuria zone, Northwest Ethiopia, 2021: a cross-sectional study. *BMJ Open*. 2023;13(1):e065169. doi:10.1136/bmjopen-2022-065169
- Tadesse T, Berhane T, Abraha TH, et al. Blood donation practice and associated factors among health professionals in Tigray regional state public hospitals, northern Ethiopia. *BMC Res Notes*. 2018;11(1):1–6. doi:10.1186/s13104-018-3786-7
- Enawgaw B, Yalew A, Shiferaw E. Blood donors' knowledge and attitude towards blood donation at North Gondar district blood bank, Northwest Ethiopia: a cross-sectional study. *BMC Res Notes*. 2019;12(1):1–6. doi:10.1186/s13104-019-4776-0
- Seid T, Dimtsu B, Zemene A, Arage G, Mossisa M, Muche T. Voluntary blood donation practice and its associated factors among HealthCare providers, at Axum Town Public Health Facilities, Northern Ethiopia: a cross sectional study. *Curr Res Complement Alternat Med*. 2017;1(2). doi:10.29011/2577-2201/100007

26. Dejene M, Tefera A, Dires A, Gedamu S, Getachew Y, Ademe S. Low blood donation practice of health sciences college students in Northeast Ethiopia: a cross-sectional study. *J Blood Med.* **2021**;12:43–51. doi:10.2147/JBM.S287398
27. Ayobami SS. Knowledge, attitude and practice of voluntary blood donation among: department of community medicine, faculty of clinical sciences, Ambrose Alli...; **2021**.
28. Mohammed AS, Yassin A, Aliyi AA. Voluntary blood donation practice and its associated factors among civil servants in Bale Robe town, Southeast Ethiopia, 2021. *SAGE Open Med.* **2022**;10:20503121221102099. doi:10.1177/20503121221102099
29. Jemberu YA, Esmael A, Ahmed KY. Knowledge, attitude and practice towards blood donation and associated factors among adults in Debre Markos town, Northwest Ethiopia. *BMC Hematol.* **2016**;16(1):1–8. doi:10.1186/s12878-016-0062-8
30. Teferi MY, Woldeesenbet SG, Feleke SA, Abebe EH, Adane HT, Alemayehu CM. Assessment of the level and factors associated with knowledge, attitude and practice of blood donation among medical and paramedical personnel in ALERT Hospital, Ethiopia. *J Public Health Res.* **2021**;10(1):1860. doi:10.4081/jphr.2021.1860
31. Tadesse T, Berhane T, Abraha TH, et al. Blood donation practice and associated factors among health professionals in Tigray regional state public hospitals, northern Ethiopia. *BMC Res Notes.* **2018**;11(1):677.
32. Feleke BE. Determinants of voluntary blood donation in the city of Bahir Dar: a case-control study. *Asian J Transfus Sci.* **2022**;16(1):56. doi:10.4103/ajts.AJTS_115_17
33. Mijena GF, Nigussie S, Gobena T, Mitiku H, Abdi H. Voluntary blood donation practice and associated factors among civil servants in Chiro Town of Western Hararghe Zone, Eastern Ethiopia: a cross-sectional study. *Res Square.* **2019**. doi:10.21203/rs.2.11580/v1
34. Darega B, Dida N, Tesfaye T, Lencha B. Voluntary blood donation practices and associated factors among regular undergraduate Madawalabu University Students, Southeast Ethiopia: a facility-based cross sectional study. *J Blood Disord Transfus S.* **2015**;5:S5.
35. Chauhan R, Kumar R, Thakur S. A study to assess the knowledge, attitude, and practices about blood donation among medical students of a medical college in North India. *J Fam Med Prim Care.* **2018**;7(4):693. doi:10.4103/jfmprc.jfmprc_54_17

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Antithrombin Deficiency and Thrombosis: A Wide Clinical Scenario Reported in a Single Institution

Ana Marco-Rico & Pascual Marco-Vera

To cite this article: Ana Marco-Rico & Pascual Marco-Vera (2023) Antithrombin Deficiency and Thrombosis: A Wide Clinical Scenario Reported in a Single Institution, Journal of Blood Medicine, , 499-506, DOI: [10.2147/JBM.S416355](https://doi.org/10.2147/JBM.S416355)

To link to this article: <https://doi.org/10.2147/JBM.S416355>



© 2023 Marco-Rico and Marco-Vera.



Published online: 01 Sep 2023.



Submit your article to this journal



Article views: 271



View related articles



View Crossmark data



Citing articles: 3 View citing articles

Antithrombin Deficiency and Thrombosis: A Wide Clinical Scenario Reported in a Single Institution

Ana Marco-Rico ^{1,2}, Pascual Marco-Vera ^{2,3}

¹Hemostasis and Thrombosis Department, Hematology Service, University General Hospital Dr. Balmis, Alicante, Spain; ²Biomedical Research Institute (ISABIAL), Alicante, Spain; ³Clinical Medicine Department, Miguel Hernández University, Alicante, Spain

Correspondence: Ana Marco-Rico, Hemostasis and Thrombosis Department, Hematology Service, University General Hospital Dr. Balmis, Pintor Baeza Avenue, 12, Alicante, 03010, Spain, Tel +34 965913863, Fax +34 965913869, Email marco_anaric@gva.es

Abstract: Congenital antithrombin (AT) deficiency represents the form of thrombophilia with the highest thrombotic risk. It is characterized by a heterogeneous clinical presentation, depending mostly on the family history of thrombosis and type of genetic mutation. Inherited AT deficiency promotes idiopathic thrombosis at an early age (even in the pediatric population) and at atypical sites. Therefore, a positive family background necessitates ruling out this high-risk thrombophilia at a young age. Studying first-degree relatives, even if they are asymptomatic, is essential to establish thromboprophylaxis and a proper therapeutic approach in case of thrombosis. Patients with congenital AT deficiency require indefinite anticoagulation owing to the high thrombotic recurrence rate. Here, we present four unrelated cases reported in our institution who were diagnosed with hereditary AT deficiency, with a contrasting clinical evolution.

Keywords: hereditary antithrombin deficiency, thrombosis, anticoagulation, heparin resistance, *SERPINC1* gene mutation

Introduction

Antithrombin (AT) is a physiological natural anticoagulant that inhibits procoagulant serine proteases, such as activated factor II, IX, X and XI.¹ In 1965, Egeberg reported the first family with AT deficiency and venous thrombosis (VT), with a decrease in both AT and heparin cofactor activity to approximately half the normal level.² A low level of AT activity disrupts the hemostatic balance and, therefore, promotes thrombosis.³

Hereditary AT deficiency is an autosomal dominant condition, with an estimated prevalence in the general population between 0.02% and 0.2%. In patients who develop venous thromboembolic disease, either VT or pulmonary embolism (PE), the prevalence varies between 1% and 5%.^{4,5} Nearly 60% of the thrombotic events are unprovoked, while the remaining 40% are associated with a transient risk factor.⁶ Hereditary AT deficiency is associated with the highest risk of thrombosis.^{4,5}

Quantitative (type I) and qualitative (type II) defects have been described. In type I, the level of AT antigen and its activity are similarly reduced,⁷ whereas in type II variants, the AT antigen level remains normal, but the AT activity is reduced as the result of a dysfunctional protein.⁸ Regarding the frequency of each type in people with inherited AT deficiency, although type II is much more frequent in the general population (12% with type I vs 88% with type II), type I is more prevalent in patients who develop thrombosis (60% with type I vs 40% with type II).⁹ This is the reason why type II could be underdiagnosed, especially if there is no family history of thrombosis (see Table 1).

The alteration of a single allele of *SERPINC1* is the most frequent genetic mutation found in AT deficiency (352 genetic variants have been described).⁸ Most of the *SERPINC1* genetic variants are point mutations (226 of 352 genetic variants). (See Table 1 for the most common mutations in the *SERPINC1* gene, depending on the type of AT deficiency.) However, other mutations that affect the N-glycosylation pathway have been reported (up to 27%). These defects (hypoglycosylation) reduce the levels and the anticoagulant activity of AT as a result of an increase in 3-N-glycan AT in plasma.¹⁰

Table I Inherited AT Deficiency: Types, Frequency of Each Type in the General Population and in People with Thrombosis, and *SERPINC1* Mutations

Types of AT Deficiency	Frequency in the General Population	Frequency in People with Thrombosis	<i>SERPINC1</i> Mutations
<u>Type I</u> – AT antigen and activity are similarly reduced	12%	60%	Single base pair substitutions Others: insertion–deletion variants Preferred regions: codons 244–245, 81, 106–107
<u>Type II</u> – AT antigen is normal – AT activity is reduced due to a dysfunctional protein	88%	40%	Most mutations: Missense gene variants
<u>Type IIa or reactive site</u> – Reactive domain is affected, preventing the binding of AT to its target protease			Most mutations occur: – At residues Ala382 and Ala384 in the hinge region of AT – Around the reactive domain at residues 392, 393 and 394
<u>Type IIb or heparin-binding site</u> – The mutation, located in the heparin-binding domain, disturbs the interaction with heparin or AT activation			Most mutations involve residues 41, 47, 99 and 129
<u>Type IIc or pleiotropic effect</u> – The mutation affects both the reactivity and heparin affinity			Most mutations involve residues 402, 404–407 and 429

Abbreviation: AT, antithrombin.

Clinical presentation may vary, depending mainly on the family history of thrombosis and type of genetic mutation. Type I AT-deficient patients usually develop thrombosis at an early age and have a tendency to recurrence. In contrast, type II AT-deficient patients have a wide clinical thrombotic presentation, ranging from mild to very severe. Of note, type IIb, or heparin-binding site mutation, carries a lower thrombotic risk.⁸

Homozygous AT deficiency type I is incompatible with life, while few cases of homozygous individuals with type IIb or mild type IIc have been described.^{8,9} Most patients with inherited heterozygous AT deficiency have an AT activity between 40% and 60%. Whether the risk of thrombosis correlates with the decrease in AT activity is still a matter of debate.⁹

The association with other additional genetic variants, such as factor V Leiden or the prothrombin G20210A mutation, increases the thrombotic risk, as does the presence of simultaneous acquired thrombotic risk factors, including immobilization, oral contraceptive intake, surgery, pregnancy, cancer and obesity.⁶

De la Morena-Barrio et al described an association between inferior vena cava atresia (IVCA) and congenital AT deficiency caused by the homozygous *SERPINC1* c.391C>T variant (p.Leu131Phe; antithrombin Budapest 3). They included 61 patients with this mutation from a total of 1118 patients with congenital AT deficiency from Spain and Hungary. More than 70% of patients with available computed tomography (CT) had an atresia of the inferior vena cava (IVC) system. The authors suggested that thrombosis in the developing fetal vessels could be the reason for this anomaly.¹¹

Additional case reports have described combined IVCA and congenital AT deficiency. According to these publications, ultrasound screening in newborns with relatives with IVCA and congenital AT deficiency is suggested, and early therapeutic intervention is necessary to prevent further thrombotic complications.^{12,13}

Age is an important risk factor for thrombosis development. Up to 60% of patients with inherited AT deficiency have thrombosis before the age of 65.¹⁴ De la Morena-Barrio et al designed a multicenter study to determine the prevalence of

thrombosis and presented clinical data in the pediatric population with inherited AT deficiency.¹⁴ The incidence of thrombosis in this population was 4.32 cases/1000 patients per year (7.5%), much higher than the incidence described in the general population (0.014%/year). They observed thrombosis at atypical sites such as the splanchnic vein, cerebral venous sinus or IVC, mainly in neonates and young children (<6 years old). Adolescents usually developed VT in lower limb and PE, and nearly half of them (47%) were provoked.¹⁴ An increased risk of first VT in AT-deficient subjects compared to controls (OR 16.26, 95% CI 9.90–26.70; $P < 0.00001$) has been reported.¹⁵ An estimated annual risk of VT of up to 2.3% (95% CI 0.2–6.5) has been described.¹⁵ In addition, the annual risk of recurrence without long-term anticoagulant therapy was 8.8% (95% CI 4.6–14.1) for AT-deficient and 4.3% (95% CI 1.5–7.9) for non-AT-deficient VT patients (but with other types of thrombophilia).¹⁶

Patients with inherited AT deficiency who develop thrombosis require indefinite anticoagulation owing to the high thrombotic recurrence rate.¹⁶ Anticoagulation with vitamin K antagonists (VKAs) is the treatment of choice. AT deficiency induces heparin resistance. Therefore, reaching a therapeutic level of anti-Xa in patients anticoagulated with heparin can be challenging, despite the administration of the appropriate heparin dose adjusted for body weight. Using AT concentrates is a feasible option, together with heparin at therapeutic doses to treat acute thrombotic episodes and prevent new ones.^{5,17} Clinical evidence on the use of direct oral anticoagulants (DOACs) in AT-deficient patients is lacking. Case studies suggest that DOACs do not seem to be less effective than VKAs. No recurrences have been reported.^{18–20}

Ten different families with hereditary AT deficiency are registered at our institution. Most of the family members require indefinite anticoagulation owing to thrombosis development.

Here, we present four unrelated patients with inherited AT deficiency who have been diagnosed and are being managed in our institution, as a reflection of the heterogeneous clinical presentation and individualized management.

Case I (C1)

Adult Woman with Venous Free-Floating Thrombus Despite Correct Anticoagulation

C1 is a 47-year-old woman diagnosed with AT deficiency type I (AT activity of 45% and AT antigen of 40%, confirmed in two different samples) in her childhood due to the known genetic background (father, aunts and cousins with AT deficiency and thrombosis). In all cases described in this manuscript, AT antigen was determined by immunoturbidimetry (Liatest ATIII®; Stago, Paris, France), while AT activity was measured using a factor II (bovine thrombin)-based chromogenic method (STA®-STACHROM® ATIII; Stago, Paris, France).

She had required indefinite anticoagulation since she developed a proximal deep VT in the left leg, confirmed by compression ultrasound, in the 15th week of pregnancy despite thromboprophylaxis with low-molecular-weight heparin (LMWH). She had no cardiovascular risk factors (CVRFs). The ultrasound performed at the 30th week of pregnancy described resolution of the thrombosis. The dose of LMWH was then adjusted to a therapeutic dose (enoxaparin 1 mg/kg every 12 hours) and anti-Xa activity was measured to ensure a therapeutic range (0.5–1.2 U/mL). The genetic analysis (detected by nanopore sequencing) showed a heterozygous mutation in c.1366G>C (p.Gly456Arg) in the *SERPINC1* gene.

After delivery, she maintained a correct anticoagulation range with VKAs (international normalized ratio [INR] range 2.0–3.0). In October 2021, C1 was diagnosed with ovarian endometrial cancer stage I according to the International Federation of Gynecology and Obstetrics (FIGO). She underwent a hysterectomy and right adnexectomy in January 2022 and required adjuvant chemotherapy with carboplatin, starting in February 2022. The follow-up CT scan revealed cancer remission. She was anticoagulated with LMWH at therapeutic doses (enoxaparin 1 mg/kg every 12 hours) while she received chemotherapy, and this was maintained until complete remission of the cancer. Anti-Xa activity was within the therapeutic range at all times. Twenty-five days after stopping chemotherapy, C1 was admitted to the hospital with moderate exercise-induced dyspnea and dry cough for 7 days. Low-load severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection was detected by polymerase chain reaction (PCR). The CT scan showed multiple filling defects related to PE and pneumonia in the right superior lung. The ultrasound of both lower limbs revealed the presence of deep proximal VT in the left leg. No IVC thrombus was shown. The INR at admission was 2.3. LMWH at therapeutic doses (enoxaparin 1 mg/kg every 12 hours) and AT replacement to achieve an AT level between 80% and 120% were administered, reaching a therapeutic anti-Xa activity range. C1 had a good clinical evolution of the pneumonia with

supplemental oxygen. She was discharged from the hospital 5 days later; the PCR was then negative for SARS-CoV-2. The INR range was adjusted to 2.5–3.5. Twenty days later, the follow-up ultrasound described the presence of a venous free-floating thrombus in the IVC. No data on IVCA were described. The INR at that moment was 2.8 and the patient remained asymptomatic. The thrombophilia study did not reveal inherited or acquired thrombophilia, except for the previously known AT deficiency. Similarly to the previous in-hospital admission, LMWH at therapeutic doses and AT supplementation were given. Owing to the difficulties in preventing new thrombosis despite correct anticoagulation, an IVC was placed. A new target INR of 3.0–4.0 was established. Three weeks later, the CT scan did not show the free-floating thrombus and the ovarian endometrial cancer remained in radiological and analytical remission. The vena cava filter was then removed. She remains currently asymptomatic and close INR monitoring is performed (INR self-monitoring).

Comments on Case I

Our patient, in agreement with type I AT deficiency, has similar AT antigen and activity levels. The recurrent thrombotic phenotype, both in the patient and in direct affected relatives, despite correct anticoagulation, also correlates with type I AT deficiency.^{7,8}

Genetic study should be considered in the case of a positive family AT-deficient background at a young age, in all first-degree relatives, even those who are asymptomatic.^{7,8} A c.1366G>C (p.Gly456Arg) heterozygous mutation in the *SERPINC1* gene has been associated with type I AT deficiency.⁶

Further treatment/prophylactic management is necessary, considering the high thrombotic risk and recurrence rate of inherited AT deficiency.¹⁴

Determination of AT activity remains the main diagnostic tool for hereditary AT deficiency. According to the literature, an AT activity below 70% overall, in the context of a positive family thrombotic history, suggests congenital AT deficiency.⁶ Despite receiving antithrombotic prophylaxis with LMWH, C1 developed her first thrombotic episode during pregnancy, which is a well-known prothrombotic setting.

It has been suggested that warfarin could increase AT levels. However, this is not consistent throughout the literature.^{21,22} Close INR monitoring is essential to ensure a therapeutic range. However, as reported in C1, despite a correct INR range, thrombosis can appear in the presence of other prothrombotic situations, such as cancer and SARS-CoV-2 infection. Cancer induces a procoagulant state and increases both arterial and venous thrombotic incidence rates (2–5% and 4–20%, respectively). In addition, chemotherapy may decrease anticoagulants and increase procoagulants, such as tissue factor, and can induce platelet activation, all together promoting thrombosis.²³ The thrombotic risk could vary depending on the anticancer agent. In particular, C1 received platin-based agents, which are known to increase thrombosis compared to non-platin combinations.²⁴ The mechanism by which platin increases thrombosis is still a matter of debate. In these patients, elevated circulating von Willebrand factor, platelet activation, increased levels of thrombin–antithrombin complexes and increased D-dimer have been described.^{25,26} Thrombosis and coagulopathy, together with pulmonary injury and inflammation, are clinically associated with coronavirus disease 2019 (COVID-19) infection. The endothelial injury associated with the cytokine storm promotes thrombosis. In addition, low ADAMTS13 activity, probably related to the excess release of von Willebrand factor, contributes to this prothrombotic COVID-19-related state.²⁶

An alternative way to prevent thrombotic recurrence in patients with INR in the therapeutic range could be to increase the optimal INR range or the administration of LMWH with close monitoring of anti-Xa activity, considering, if necessary, AT concentrates to control heparin resistance.²⁷

Our patient developed a new severe thrombotic event despite a proper anticoagulation INR range (INR of 2.8). In that setting, to avoid further episodes, a vena cava filter was recommended. An IVC filter is indicated in patients with VT and contraindication to anticoagulation, failure of anticoagulation or VT progression despite adequate anticoagulation.²⁸ Once the venous free-floating thrombus disappears, the vena cava filter can be removed, and a strict INR control to ensure correct anticoagulation should be performed.

Case 2 (C2)

Massive Cerebral Vein Thrombosis in Childhood

C2 is a 5-year-old girl admitted to a local hospital with sudden tetraparesis with light predominance in her right hemibody and Broca aphasia. Her medical records included an AT deficiency type I (AT activity of 40% and AT antigen of 35%, confirmed in two different samples). A cousin had suffered from cerebral venous thrombosis (CVT) 3 days after birth, her father had developed a deep VT in childhood, and an asymptomatic aunt had been previously diagnosed with inherited AT deficiency. The genetic analysis showed a heterozygous mutation in c.481C>T (p.Arg161Ter) in the *SERPINC1* gene.

Hemodynamic stability was achieved during admission to the intensive care unit. An AT activity of 49% was confirmed at admission. A generalized paresthesia, more noticeable in the right upper extremity, was observed. Initial contrast brain CT revealed filling defects affecting the internal cerebral veins, Galeno vein, inferior longitudinal sinus and left transverse sinus, with extension to the left internal jugular vein, suggesting deep CVT. AT replacement to maintain AT levels of 100%, in addition to LMWH (enoxaparin 1 mg/kg twice per day), was immediately initiated. However, the intracranial pressure increased, and clinical worsening was evident, with the presence of seizures and posterior loss of consciousness, probably secondary to obstructive hydrocephalus. Orotracheal intubation was required. Mechanical thrombectomy was then carried out and a partial recanalization was achieved. A 4 mg bolus of local alteplase was injected in the straight sinus, followed by a continuous alteplase infusion at a dose of 1 mg per hour. AT replacement and LMWH 1 mg/kg every 12 hours were administered, reaching AT levels of 100% and an anti-Xa activity in the therapeutic range. The patient experienced a gradual neurological improvement, coinciding with a radiological reduction and posterior resolution of the massive thrombosis and edema. She is currently on anticoagulation (INR target 2.5–3.5) and asymptomatic.

Comments on Case 2

CVT is an uncommon but severe condition, more frequently affecting young children.⁹ Although high-risk inherited thrombophilia has been related to common sites of thrombosis such as deep veins in the legs, an association with rare presentations has been described.¹⁴

More than half of the patients with AT deficiency develop a spontaneous thrombosis.^{5,8} CVRFs should be controlled to minimize thrombotic risk, and antithrombotic prophylaxis with heparin should be considered in high-risk prothrombotic situations.⁶ In our patient, there was no trigger associated with thrombosis. Of note, her younger cousin developed a CVT 3 days after birth.

AT replacement may be adequate in an acute VT, especially in unusual severe thrombosis, despite adequate anticoagulation and heparin resistance.^{6,17} Our patient was, consequently, the optimal candidate for both anticoagulation at therapeutic doses and AT replacement, owing to the severity and atypical presentation.

In CVT, endovascular thrombolysis with or without mechanical thrombectomy may be beneficial in patients with poor prognosis despite anticoagulant treatment with heparin, due mainly to the impossibility of dissolving the extensive thrombus. However, this is based on case series and not on controlled randomized trials.²⁹ Siddiqui et al included a systematic review of 185 patients undergoing mechanical thrombectomy, and a mean recanalization of 95% was obtained. These data were obtained from small retrospective studies.³⁰

The mutation described in C2 and her relatives is associated with hereditary quantitative AT deficiency (type I) and carries a high thrombotic risk.³¹ In fact, all affected relatives except for one aunt developed thrombosis at a very young age. A peak incidence of thrombosis, especially CVT during the neonatal period, has been described in hereditary AT-deficient patients.³² The physiological AT deficiency at birth is exacerbated by the hereditary defect of AT, inducing prothrombotic situations such as hypoxia and the release of tissue factor more easily, together with possible trauma and manipulation during delivery.³²

Case 3 (C3)

Female Adult with Neither Familiar nor Personal Thrombosis and Congenital AT Deficiency

C3 is a 47-year-old businesswoman, a habitual smoker of 10 cigarettes per day for 15 years, with no other additional CVRFs. No family history of thrombosis was reported. In April 2001, in the setting of three miscarriages before the 10th week of pregnancy, a thrombophilia study was performed. A type II AT deficiency was detected, confirmed in two different samples (AT activity of 46%, AT antigen of 80%). Family screening was completed, and her father, uncle and grandfather had similar AT levels (activity and antigen) to C3. The genetic analysis showed a heterozygous mutation in c.235C>T (p.Arg79Cys) in the *SERPINC1* gene. None of them had previously developed thrombosis. C3 is currently taking hormonal replacement with transdermal estrogen owing to early menopause.

Comments on Case 3

Despite the high thrombotic risk associated with hereditary AT deficiency, there may be adult patients with known underlying AT deficiency and no previous thrombotic events. This uncommon scenario could happen mainly in patients with no CVRFs and a healthy lifestyle. Up to 40% of patients with hereditary AT deficiency had thrombosis associated with a transient risk factor.⁶ C3, except for smoking, has an active healthy lifestyle, as do her first-degree affected relatives. They should be given prophylactic heparin during a prothrombotic situation, such as immobilization, pregnancy or active cancer. Hormonal treatment containing a high estrogen dose is not recommended, as it could increase the thrombotic risk. Low-dose estrogen pills, pills containing only progestagen or an intrauterine device are treatments of choice in high-risk prothrombotic women who require hormonal treatment.³² In postmenopausal women, transdermal estrogen does not increase thrombotic risk.³³ The most suitable option should be carefully considered by the gynecologist, depending on the reason for hormonal treatment and the presence of other CVRFs.^{32,33}

The genetic mutation could play a role in the severity of thrombosis. The heterozygous missense mutation, a cytosine to thymine substitution at nucleotide position 235 in exon 2 of the *SERPINC1* gene (p.Arg79Cys), has been associated with a low prevalence of thrombosis. This is a mutation in the heparin binding site, described in type IIb AT deficiency. Subjects with this mutation, mainly heterozygous patients, rarely develop thrombosis.³² Alhenc-Gelas et al did not describe an increase in the endogenous thrombin potential in type IIb heterozygous patients compared to a control group without AT deficiency (2142 nMxmin vs 2299 nMxmin), being associated with a low risk of VT.³⁴ Yoo et al reported the first patient with this heterozygous mutation who had deep VT and a family history of thrombosis.³⁵

In case antithrombotic therapy with heparin is needed, patients with heparin binding site mutation may require AT replacement, as heparin itself could not generate an appropriate antithrombotic effect.^{6,17}

Case 4 (C4)

Adult Man with Recurrent Thrombosis Despite Correct Anticoagulation

C4 is a 64-year-old man who requires indefinite anticoagulation owing to recurrent PE. No family history of thrombophilia was reported. The first PE and deep VT in his right leg occurred in 2000 spontaneously at the age of 46. He had, at that time, several CVRFs, including hypertension, dyslipidemia and obesity (body mass index of 33 kg/m²), and he had been a smoker of 15 cigarettes per day for 15 years. He was being followed by the general practitioner owing to hepatic steatosis. Anticoagulation with VKAs was then initiated, reaching the INR target of 2.5 easily. The CVRFs significantly improved as a result of a healthy lifestyle and proper medication for hypertension and dyslipidemia. An AT activity of 65% was detected, without any other thrombophilia defects. This mild AT deficiency was not considered of relevance and was first reported in the low range of normality (in our laboratory, the normal levels for AT activity range between 70% and 100%). However, in further routine analytical controls, AT activity remained between 60% and 65% and the AT antigen was 53%, confirmed in two different samples. Therefore, in the setting of an idiopathic thrombosis and the previous analytical abnormalities, AT deficiency type I was confirmed. The patient had a heterozygous point mutation in the *SERPINC1* gene, c.1219-3C>A, affecting intron 6, very close to the exon 6 donor sequence (IVS6-3C>A). Four years later, the patient was admitted to the emergency department with idiopathic recurrent PE despite a correct INR (2.4). He had a favorable clinical evolution with

LMWH at therapeutic doses (enoxaparin 1 mg/kg every 12 hours) and AT concentrates to reach an AT activity of 100%. At hospital discharge, the INR range was adjusted to 2.5–3.5. No further thrombotic events have been reported.

Comments on Case 4

Similarly to C3, in a few families AT deficiency is not diagnosed at an early age because of the absence of personal or family thrombotic history. C4 developed his first thrombotic event at the age of 46 in the setting of different CVRFs. The mild AT activity deficiency was first considered to lie in the range of normality. This result correlated with the diagnosis of hepatic steatosis included in his medical records. However, the rest of the proteins of hepatic origin included in the thrombophilia study, such as protein S, protein C and plasminogen, were strictly normal, as was the hepatic function profile (transaminases and bilirubin). Later, once AT type I deficiency had been confirmed, the genetic family study was performed. His older sister did not carry the mutation and had a normal AT activity (90%), while his parents were not available for the genetic study. C4 has been on anticoagulation since this first episode owing to high recurrent thrombotic rate.¹⁶ Strict INR monitoring is essential to prevent further thrombotic episodes, ensuring no INR below the target range. In addition, appropriate control of CVRFs is necessary to reduce the thrombotic risk. C4 achieved a substantial improvement in his CVRFs with medication for hypertension and dyslipidemia, and having lost weight. An appropriate level of anticoagulation was achieved, with no INR fluctuations. However, he developed a new thrombosis that required INR target adjustment.

The mutation detected in the *SERPINC1* gene could affect the appropriate processing of exon/intron 6 during mRNA formation, and it is associated with high thrombotic risk.³⁶ This mutation has been described previously.^{36,37}

Conclusions

- Inherited AT deficiency is the most severe type of thrombophilia, with a remarkable clinical heterogeneity that requires indefinite anticoagulation.
- Genetic testing should be considered in all first-degree relatives at a young age, and appropriate treatment/prophylactic management is necessary to minimize thrombotic risk.
- Type IIb confers a lower thrombotic risk and may be underdiagnosed.

Ethics and Consent

The study participants have given written informed consent to participate and for publication of the data. Parental consent was obtained for the case of a minor. Institutional approval was not required for publication.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Rosenberg RD, Damus PS. The purification and mechanism of action of human antithrombin-heparin cofactor. *J Biol Chem*. 1973;248(18):6490–6505. doi:10.1016/S0021-9258(19)43472-8
2. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. *Thromb Diath Haemorrh*. 1965;13:516–530.
3. Tsuchida T, Hayakawa M, Kawahara S, Kumano O. Thrombin generation capacity is enhanced by low antithrombin activity and depends on the activity of the related coagulation factors. *Thromb J*. 2022;20(1). doi:10.1186/s12959-022-00388-w
4. Colucci G, Tsakiris D. Thrombophilia screening revisited: an issue of personalized medicine. *J Thromb Thrombolysis*. 2020;49(4):618–629. doi:10.1007/s11239-020-02090-y
5. Martinelli I, Mannucci PM, De Stefano V, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. *Blood*. 1998;92(7):2353–2358. doi:10.1182/blood.V92.7.2353
6. Pabinger I, Thaler J. How I treat patients with hereditary antithrombin deficiency. *Blood*. 2019;134(26):2346–2353. doi:10.1182/blood.2019002927
7. Bravo-Pérez C, Toderici M, Chambers JE, et al. Full-length antithrombin frameshift variant with aberrant C-terminus causes endoplasmic reticulum retention with a dominant-negative effect. *JCI Insight*. 2022;7(19):e161430. doi:10.1172/jci.insight.161430
8. Bravo-Perez C, De la Morena Barrio ME, Vicente V, Corral J. Antithrombin deficiency as a still underdiagnosed type of thrombophilia: a primer for internists. *Pol Arch Intern Med*. 2020;130(10):868–877. doi:10.20452/pamw.15371
9. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. *Haemophilia*. 2008;14(6):1229–1239. doi:10.1111/j.1365-2516.2008.01830.x
10. De la Morena-Barrio ME, Martinez-Martinez I, de Cos C, et al. Hypoglycosylation is a common finding in antithrombin deficiency in the absence of a *SERPINC1* gene defect. *J Thromb Haemost*. 2016;14(8):1549–1560. doi:10.1111/jth.13372

11. De la Morena-Barrio ME, Gindele R, Bravo-Pérez C, et al. High penetrance of inferior vena cava system atresia in severe thrombophilia caused by homozygous antithrombin Budapest 3 variant: description of new syndrome. *Am J Hematol*. 2021;96(11):1363–1373. doi:10.1002/ajh.26304
12. Muller-Knapp M, Classen CF, Knoefler R, et al. Coexistence of antithrombin deficiency and suspected inferior vena cava atresia in an adolescent and his mother: case report and clinical implications. *Thromb J*. 2021;19:105–112. doi:10.1186/s12959-021-00360-0
13. Lukaseder J, Feldman R, Steiner A. Recurrent vein thrombosis with agenesis of the inferior vena cava and AT III deficiency. *Phlebologie*. 2017;46(01):31–33. doi:10.12687/phleb2345-1-2017
14. De la Morena-Barrio B, Orlando C, de la Morena-Barrio ME, Vicente V, Jochmans K, Corral J. Incidence and features of thrombosis in children with inherited antithrombin deficiency. *Haematologica*. 2019;104(12):2512–2518. doi:10.3324/haematol.2018.210666
15. Di Minno MND, Ambrosino P, Ageno W, Rosendaal F, Di Minno G, Dentali F. Natural anticoagulants deficiency and the risk of venous thromboembolism: a meta-analysis of observational studies. *Thromb Res*. 2015;135(5):923–932. doi:10.1016/j.thromres.2015.03.010
16. Croles FN, Borjas-Howard J, Nasserinejad K, Leebeek FW, Meijer K. Risk of venous thrombosis in antithrombin deficiency: a systematic review and Bayesian meta-analysis. *Semin Thromb Hemost*. 2018;44:315–326. doi:10.1055/s-0038-1625983
17. Bauer KA, Nguyen-Cao TM, Spears JB. Issues in the diagnosis and management of hereditary antithrombin deficiency. *Ann Pharmacother*. 2016;50(9):758–767. doi:10.1177/1060028016651276
18. Elsebaie MAT, van Es N, Langston A, Büller HR, Gaddh M. Direct oral anticoagulants in patients with venous thromboembolism and thrombophilia: a systematic review and meta-analysis. *J Thromb Haemost*. 2019;17(4):645–656. doi:10.1111/jth.14398
19. Soerajja Bhoelan B, Mulder R, Lukens MV, Meijer K. Direct oral anticoagulants in antithrombin deficiency: initial experience in a single center. *Thromb Haemost*. 2021;121(02):242–245. doi:10.1055/s-0040-1715647
20. Campello E, Spiezia L, Simion C, et al. Direct oral anticoagulants in patients with inherited thrombophilia and venous thromboembolism: a prospective study. *J Am Heart Assoc*. 2020;9(23):e018917. doi:10.1161/JAHA.120.018917
21. O'Brien JR, Etherington MD. Effect of heparin and warfarin on antithrombin III. *Lancet*. 1977;2(8050):1231.
22. Sanfelippo MJ, Engel JM, Onitilo AA. Antithrombin levels are unaffected by warfarin. *Arch Pathol Lab Med*. 2014;138(7):967–968. doi:10.5858/arpa.2013-0065-OA
23. Grover SP, Hisada YM, Kasthuri RS, Reeves BN, Mackman N. Cancer therapy-associated thrombosis. *Arterioscler Thromb Vasc Biol*. 2021;41(4):1291–1305. doi:10.1161/ATVBAHA.120.314378
24. Moik F, van Es N, Posch F, et al. Gemcitabine and platinum-based agents for the prediction of cancer associated venous thromboembolism: results from the Vienna cancer and thrombosis study. *Cancers*. 2020;12(9):2493. doi:10.3390/cancers12092493
25. Seng S, Liu Z, Chiu SK, et al. Risk of venous thromboembolism in patients with cancer treated with Cisplatin: a systematic review and meta-analysis. *J Clin Oncol*. 2012;30(35):4416–4426. doi:10.1200/JCO.2012.42.4358
26. Marco A, Marco P. Von Willebrand factor and ADAMTS13 as clinical severity markers in patients with COVID-19. *J Thromb Thrombolysis*. 2021;52(2):497–503. doi:10.1007/s11239-021-02457-9
27. Luxembourg B, Pavlova A, Geisen C, et al. Impact of the type of *SERPINC1* mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. *Thromb Haemost*. 2014;111(2):249–257. doi:10.1160/TH13-05-0402
28. Li X, Haddadin I, McLennan G, et al. Inferior vena cava filter: comprehensive overview of current indications, techniques, complications and retrieval rates. *Vasa*. 2020;49(6):449–462. doi:10.1024/0301-1526/a000887
29. Ferro JM, Boussier GM, Canhao P, et al. European Stroke Organization Guidelines for the diagnosis and treatment of cerebral venous thrombosis. *Europ Stroke Journal*. 2017;2(3):195–221. doi:10.1177/2396987317719364
30. Siddiqui FM, Dandapat S, Banerjee C, et al. Mechanical thrombectomy in cerebral venous thrombosis: systematic review of 185 cases. *Stroke*. 2015;46(5):1263–1268. doi:10.1161/STROKEAHA.114.007465
31. Gandrille S, Vidaud D, Emmerich J, et al. Molecular basis for hereditary antithrombin III quantitative deficiencies: a stop codon in exon IIIa and a frameshift in exon VI. *Br J Haematol*. 1991;78(3):414–420. doi:10.1111/j.1365-2141.1991.tb04457.x
32. Abou-Ismaïl MY, Sridhar DC, Nayak L. Estrogen and thrombosis: a bench to bedside review. *Thromb Res*. 2020;192:40–51. doi:10.1016/j.thromres.2020.05.008
33. Canonico M, Oger E, Plu-Bureau G, et al. Hormone therapy and venous thromboembolism among postmenopausal women. *Circulation*. 2007;115(7):840–845. doi:10.1161/CIRCULATIONAHA.106.642280
34. Alhenc-Gelas M, Canonico M, Picard V. Influence of natural *SERPINC1* mutations on ex vivo thrombin generation. *J Thromb Haemost*. 2010;8(4):845–848. doi:10.1111/j.1538-7836.2010.03750.x
35. Yoo JH, Maeng HY, Kim HJ, Lee KA, Choi JR, Song J. A heparin binding site Arg79Cys missense mutation in the *SERPINC1* gene in a Korean patient with hereditary antithrombin deficiency. *Ann Clin Laboratory Sci*. 2011;41:89–92.
36. De la Morena Barrio ME, López-Gálvez R, Martínez-Martínez I, et al. Defects of splicing in antithrombin deficiency. *Res Pract Thromb Haemost*. 2017;1(2):216–222. doi:10.1002/rth2.12025
37. Human Gene Mutation Database (HGMD) [homepage on the Internet]; 2023. Available from: www.hgmd.cf.ac.uk. Accessed August 24, 2023.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Autoimmune Hemolytic Anemia Caused by Cold Agglutinin Antibodies in Systemic Lupus erythematosus—a Rare Association: Case Report

Luis Miguel Osorio-Toro, Jhon Herney Quintana-Ospina, Luis Álvaro Melo-Burbano, Paola Andrea Ruiz-Jiménez, Jorge Enrique Daza-Arana, Giovanna Patricia Rivas-Tafurt & Jorge Hernán Izquierdo-Loaiza

To cite this article: Luis Miguel Osorio-Toro, Jhon Herney Quintana-Ospina, Luis Álvaro Melo-Burbano, Paola Andrea Ruiz-Jiménez, Jorge Enrique Daza-Arana, Giovanna Patricia Rivas-Tafurt & Jorge Hernán Izquierdo-Loaiza (2023) Autoimmune Hemolytic Anemia Caused by Cold Agglutinin Antibodies in Systemic Lupus erythematosus—a Rare Association: Case Report, Journal of Blood Medicine, , 507-511, DOI: [10.2147/JBM.S420937](https://doi.org/10.2147/JBM.S420937)

To link to this article: <https://doi.org/10.2147/JBM.S420937>



© 2023 Osorio-Toro et al.



Published online: 04 Sep 2023.



Submit your article to this journal



Article views: 358



View related articles



View Crossmark data



Citing articles: 4 View citing articles

Autoimmune Hemolytic Anemia Caused by Cold Agglutinin Antibodies in Systemic Lupus erythematosus—a Rare Association: Case Report

Luis Miguel Osorio-Toro¹⁻³, Jhon Herney Quintana-Ospina¹⁻³, Luis Álvaro Melo-Burbano¹⁻³, Paola Andrea Ruiz-Jiménez¹⁻³, Jorge Enrique Daza-Arana^{1,4}, Giovanna Patricia Rivas-Tafurt^{1,2}, Jorge Hernán Izquierdo-Loaiza^{1,2}

¹Specialization in Internal Medicine, Department of Health, Universidad Santiago de Cali, Santiago de Cali, Colombia; ²Department of Research and Education, Clínica de Occidente S.A, Santiago de Cali, Santiago de Cali, Colombia; ³Genetics, Physiology, and Metabolism Research Group (GEFIME), Universidad Santiago de Cali, Santiago de Cali, Colombia; ⁴Health and Movement Research Group, Universidad Santiago de Cali, Santiago de Cali, Colombia

Correspondence: Jorge Enrique Daza-Arana, Specialization Program in Internal Medicine, Department of Health, Universidad Santiago de Cali, Calle 5 # 62-00, Santiago de Cali, Colombia, Tel +57 3108923676, Fax +57 3108923676, Email jorgedaza.epidemiologia@gmail.com

Abstract: Autoimmune hemolytic anemias (AIHAs) are rare and heterogeneous disorders characterized by the destruction of red blood cells by warm or cold antibodies. Hemolytic anemia associated with warm antibodies is the most common, whereas cold antibodies are rare and infrequent in cases published in the scientific literature. Herein, we present the case of a young patient with systemic lupus erythematosus (SLE) and autoimmune hemolytic anemia caused by cold antibodies. Initially, infectious etiology and hematological malignancy were considered, which were ruled out. She required management in the intensive care unit due to severe hematological involvement and responded well to immunomodulatory therapy. This case illustrates the importance of a strong clinical suspicion of AIHA due to cold agglutinins associated with SLE when faced with similar clinical symptoms in order to achieve a timely diagnosis and provide optimal therapy.

Keywords: autoimmune hemolytic anemia, systemic lupus erythematosus, autoimmune diseases, antibodies

Introduction

Autoimmune hemolytic anemias (AIHAs) are rare and heterogeneous disorders characterized by the destruction of red blood cells by warm or cold antibodies.¹ Cold-antibody autoimmune hemolytic anemia, also known as cold agglutinin disease when it is primary, is a type of hemolysis caused by immunoglobulin M (IgM) antibodies directed against the polysaccharide antigens of the red blood cell membrane,² accounting for 15%–30% of AIHAs.³ The red blood cell-associated glycan that is the target of cold agglutinin antibodies are those containing N-polygalactosamine.⁴ Cold agglutinin syndrome is a hemolytic disorder caused by cold antibodies; however, it arises as a result of another clinical disease, with systemic lupus erythematosus (SLE) being an uncommon cause.⁵ SLE is a multisystem disease with several clinical presentations,⁶ including hemolytic anemia, which affects less than 10% of patients and is generally caused by warm IgG antibodies. It is rare to detect hemolytic anemia mediated by cold antibodies in this disease,⁷ and the literature contains only a few descriptions of cases of hemolytic anemia caused by cold antibodies in SLE.⁷⁻⁹ As a result of the rare association between these two entities, our report is significant.

Case Presentation

A 22-year-old female patient with no relevant medical history was admitted to the emergency room of a third-level clinic for clinical symptoms that began 10 days before admission, including intermittent fever up to 39°C, general malaise, headache, myalgia, a mild self-limiting rash episode, diarrhea, and occasional emesis. On physical examination, the patient had mild pallor, icteric sclerae, supraclavicular and cervical adenopathies, synovitis in bilateral metacarpophalangeal joints, and grade II

edema in the lower limbs. Paraclinical testing was performed (Table 1), and infectious etiology and hematological neoplasia were ruled out. The classification criteria for SLE were met.¹⁰ The patient was anti-DNA negative, a kidney biopsy was recommended because of the presence of proteinuria, which was consistent with class II mesangial lupus nephritis.

Autoimmune hemolytic anemia was also confirmed, with laboratory data revealing a cold agglutinin titer Positive dilution 1/128 at 4°C, hemoglobin 6.2 g/dl, hematocrit 18.1% and monospecific direct antiglobulin strongly positive for C3d and

Table 1 Clinical Laboratory Findings

Variable	Admission	Reference Range
Antinuclear Antibodies	Positive 1/320 Homogeneous Pattern	Negative: Less than 1:160
Anti-DNA antibodies	Negative	Negative: Titer less than 1:10
Erythrocyte sedimentation rate (mm/H)	105	0–20
C-reactive protein (mg/L)	20.50	0–10
Anti-RNP (U)	41,60	Negative: 0–20
Anti-Sm (U)	22,74	Negative: 0–20
Anti-La / SSB (U)	2,00	Negative: 0–20
Anti-Ro / SSA (U)	29,27	Negative: 0–20
C3 (mg/dl)	57,70	84–160
C4 (mg/dl)	7,00	12–36
Direct Coombs test	Positive +++	Negative
Fractionated monospecific direct Coombs test	Positive c3d Negative IgG	Negative
Cold agglutinins	Positive dilution 1/128 at 4°C	Negative
Haptoglobin (mg/dl)	<8	14–258
Total bilirubin (mg/dl)	2,25	< 1.2
Indirect bilirubin (mg/dl)	1,85	< 0.7
Lactate dehydrogenase (U/L)	1438	208–378
Reticulocytes (%)	6,50	0,5–2,5
Leukocytes (cells/uL)	2.270	4.500–11.000
Neutrophils (cells/uL)	1.140 (50,5%)	55–70
Lymphocytes (cells/uL)	640 (28,1%)	17–45
Monocytes (cells/uL)	210 (9,4%)	3–14
Eosinophils (cells/uL)	10 (0,2%)	1–5
Basophils (cells/uL)	20 (0,9%)	0–2
Hemoglobin (g/dl)	6.2	12.3–15.3
Hematocrit (%)	18.1	36–45
Mean corpuscular volume (fL)	84.2	80–96
Mean corpuscular hemoglobin (pg)	29	27–33
Platelets (cells/uL)	59.000	150.000–450.000

negative for IgG, leading to the diagnosis of cold agglutinin syndrome.¹¹ The patient was transferred to the intensive care unit because of severe hematological involvement, as demonstrated by pancytopenia. Treatment was initiated with dexamethasone at a dose of 40 mg intravenously daily for 4 days and hydroxychloroquine at a dose of 200 mg daily orally, and the three cell lines showed clinical improvement and hematological recovery without the need for blood transfusions. Renal function remained stable and I did not present any other complications. The patient was discharged from the hospital after receiving outpatient treatment with prednisone at a dose of 5 mg daily orally, hydroxychloroquine at a dose of 200 mg daily orally and mycophenolate mofetil at a dose of 500 mg every 12 hours for one week and thereafter at a dose of 500 mg every 8 hours. She made steady progress to her rheumatology follow-up in the outpatient department with no new relapses.

In this case report, all procedures were performed according to the ethical and bioethical standards of the Scientific Committee of the Clínica de Occidente and the Declaration of Helsinki of 1964 and its later versions. Informed consent was obtained from the study subject prior to her participation and the publication of this study.

Discussion

Autoimmune hemolytic anemia is a rare disease with an annual incidence of 1–3 individuals out of every 100,000.¹² AIHA occurs in 10% of patients with SLE and is generally associated with warm antibodies; cold hemagglutinin disease has also been rarely observed. This paper describes the case of a patient with autoimmune hemolytic anemia due to cold agglutinins in the context of de novo SLE.

Cold hemagglutination and its association with hemolysis were described for the first time in 1903. The binding of antibodies to red blood cells activates the classical pathway of the complement system, which leads to the formation of the membrane attack complex and intravascular hemolysis. Furthermore, if the classical pathway fails, erythrocytes are opsonized with complement proteins (particularly C3b and C4b), which increase phagocytosis in the liver and spleen, leading to extravascular hemolysis.⁷ Haptoglobin levels were low in our patient, suggesting intravascular hemolysis.

The anti-erythrocyte antibodies in SLE are mainly warm IgG, but mixed AIHA has also been reported. Our case had characteristics of cold IgM AIHA.⁹ Cold agglutinin AIHA can be primary (cold agglutinin disease) or secondary (cold agglutinin syndrome) to infections, malignancies, or autoimmune conditions.¹³ Secondary etiologies other than SLE were ruled out in our case.

Corticosteroids result in remission in less than 20% of patients with cold agglutinin disease, and unacceptably high maintenance doses are often required to achieve a sustained response in the few responders. Thus, corticosteroid-sparing alternatives should be considered, with bendamustine or rituximab being the preferred treatments for these patients.¹⁴ However, in cold agglutinin syndrome, as in the case of our patient, the only established therapy is the treatment of the underlying disease.¹⁵ In our case, corticosteroids were administered and a favorable clinical and paraclinical response was observed, as reported in other similar cases in the literature with an adequate response.^{7–9}

The cases described in the literature are female, generally all in the second and third decades of life; only one case was described in a female patient in the fifth decade of life (case 2). In no case was there evidence of a history of comorbidities prior to diagnosis. Most cases with short duration of the disease. Case 1, case 3 and our case presented hypocomplementemia, which spoke of disease activity. We do not have information on the complement levels of case 2. All cases have elevated bilirubin levels at the expense of the indirect. The only case that showed renal compromise due to SLE up to the time of diagnosis was ours. All received immunosuppressive treatment with a good response, only case 2 received biologics with a good haematological response (Table 2).

Table 2 Cases of Hemolytic Anemia Caused by Cold Agglutinin Antibodies Reported in the Literature

Data	Case 1 ⁷	Case 2 ⁸	Case 3 ⁹	Index Case
Age	27 years	42 years	17 years	22 years
Sex	Female	Female	Female	Female
Disease duration	< 1 year	< 1 year	< 1 year	< 1 year

(Continued)

Table 2 (Continued).

Data	Case 1 ⁷	Case 2 ⁸	Case 3 ⁹	Index Case
Laboratory results				
Hemoglobin (g/dL)	8.3	Does not specify	5.1	6.2
Leukocytes (cells/uL)	12,400	6000	9,000	2,000
Platelets (cells/uL)	Does not specify	Does not specify	244,000	59,000
Creatinine (mg/dL)	1.1	Does not specify	0.7	0.9
Albumin (gr/dL)	2.3	Does not specify	Does not specify	4.5
ESR (mm/H)	100	Does not specify	Does not specify	105
CRP (mg/L)	12.7	Does not specify	Does not specify	20.5
BT / BI (mg/dL)	1.5 / 1.2	3.6/3.1	1.09/ 1	2.25/1.85
LDH (U/L)	586	Does not specify	1052	1438
Immune laboratory values				
C3, (mg/dL)	53	Does not specify	76 (Low)	57.70 (Low)
C4, (mg/dL)	10	Does not specify	< 7.3 (Low)	7.00 (Low)
Anti-dsDNA, n (%)	90 IU/mL (+)	11 IU/mL (+)	Positive	Negatives
LA, n (%)	Does not specify	Does not specify	Does not specify	Negatives
aCL-IgG, n (%)	Does not specify	Does not specify	Does not specify	Negatives
aCL-IgM, n (%)	Does not specify	26 U/mL (+)	< 10 U/mL	Negatives
Anti-B2-GP I IgG, n (%)	Does not specify	Does not specify	Does not specify	Negatives
Anti-B2-GP I IgM, n (%)	Does not specify	Does not specify	Does not specify	Negatives
Cold agglutinin antibodies	1:0.7000	Cold IgM agglutinin titer level.	Positives	Positive dilution 1/128 at 4°C
Organ compromise	Does not specify	Does not specify	Does not specify	Class II mesangial lupus nephritis
Comorbidities	No	No	No	No
Treatment	Prednisone	Rituximab	Methylprednisolone IV	Dexamethasone + hydroxychloroquine + mycophenolate mofetil

Conclusion

AIHA due to cold agglutinins associated with SLE is extremely rare, it is key to know other entities that lead to confusion in the final diagnosis of this pathology, such as lymphoproliferative and infectious disorders. Given the focus on different treatment regimens, the difference between cold agglutinin syndrome and the disease should be clear, since in the syndrome the treatment focuses on the management of the underlying disease. Our patient responded strongly to management with dexamethasone, hydroxychloroquine and mycophenolate.

Abbreviations

AIHA, autoimmune hemolytic anemia; SLE, systemic lupus erythematosus; IgM, immunoglobulin M.

Acknowledgments

The research team's activities were sponsored by Clínica de Occidente S.A. and the Universidad Santiago de Cali. However, the authors declare full autonomy when conducting all phases of the study.

Funding

This research has been funded by Dirección General de Investigaciones of Universidad Santiago de Cali under call No. 02-2023.

Disclosure

Dr Giovanna Patricia Rivas-Tafurt reports personal fees from Amgen and Bristol Myers Squibb, outside the submitted work. The authors report no other conflicts of interest in this work.

References

1. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. *Blood Rev.* 2020;41:100648. doi:10.1016/j.blre.2019.100648
2. Velasco Montes J, Oriñuela González I, Sanjuan López AZ, Ortiz de Zárate Ibarra Z. Anemia hemolítica por aglutininas frías y cáncer de origen desconocido [Cold agglutinin disease and cancer of unknown origin]. *Rev Esp Geriatr Gerontol.* 2010;45(3):172–173. doi:10.1016/j.regg.2009.12.002
3. Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. *Br Med J.* 1981;282(6281):2023–2027. doi:10.1136/bmj.282.6281.2023
4. Bua RO, Messina A, Sturiale L, Barone R, Garozzo D, Palmigiano A. N-Glycomics of Human Erythrocytes. *Int J Mol Sci.* 2021;22(15):8063. doi:10.3390/ijms22158063
5. Berentsen S, Tjønnfjord GE. Diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. *Blood Rev.* 2012;26(3):107–115. doi:10.1016/j.blre.2012.01.002
6. Rivas-Larrauri F, Yamazaki-Nakashimada MA. Systemic lupus erythematosus: ¿Is it one disease? *Reumatol Clin.* 2016;12(5):274–281. doi:10.1016/j.reuma.2016.01.005
7. Srinivasan N, Oswal A, Garg S, Nahar J, Gosmonova A, Nahar R. Cold agglutinin induced hemolysis in a newly diagnosed systemic lupus erythematosus. *Am J Med Sci.* 2010;339(3):270–273. doi:10.1097/MAJ.0b013e3181ac3bd5
8. Chaubey VK, Chhabra L. Cold agglutinin-induced haemolysis in association with antinuclear antibody-negative SLE. *BMJ Case Rep.* 2013;2013(jun10 1):bcr2013009337–bcr2013009337. doi:10.1136/bcr-2013-009337
9. Mohanty B, Ansari MZ, Kumari P, Sunder A. Cold agglutinin-induced hemolytic anemia as the primary presentation in SLE - A case report. *J Family Med Prim Care.* 2019;8(5):1807–1808. doi:10.4103/jfmpc.jfmpc_298_19
10. Aringer M, Petri M. New classification criteria for systemic lupus erythematosus. *Curr Opin Rheumatol.* 2020;32(6):590–596. doi:10.1097/BOR.0000000000000740
11. Berentsen S. New Insights in the Pathogenesis and Therapy of Cold Agglutinin-Mediated Autoimmune Hemolytic Anemia. *Front Immunol.* 2020;11:590. doi:10.3389/fimmu.2020.00590
12. Michel M. Classification and therapeutic approaches in autoimmune hemolytic anemia: an update. *Expert Rev Hematol.* 2011;4(6):607–618. doi:10.1586/ehm.11.60
13. Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica.* 2006;91(4):460–466.
14. Berentsen S. How I treat cold agglutinin disease. *Blood.* 2021;137(10):1295–1303. doi:10.1182/blood.2019003809
15. Berentsen S, Barcellini W. Autoimmune Hemolytic Anemias. *N Engl J Med.* 2021;385(15):1407–1419. doi:10.1056/NEJMra2033982

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Acute Basophilic Leukemia Arising from Chronic Myeloid Leukemia with +8, I(17q)(q10) and der(22)t(9;22) After Imatinib Therapy

Peng Shan, Hang Dong & Shilan Li

To cite this article: Peng Shan, Hang Dong & Shilan Li (2023) Acute Basophilic Leukemia Arising from Chronic Myeloid Leukemia with +8, I(17q)(q10) and der(22)t(9;22) After Imatinib Therapy, Journal of Blood Medicine, , 513-517, DOI: [10.2147/JBM.S412837](https://doi.org/10.2147/JBM.S412837)

To link to this article: <https://doi.org/10.2147/JBM.S412837>



© 2023 Shan et al.



Published online: 06 Sep 2023.



Submit your article to this journal



Article views: 178



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Acute Basophilic Leukemia Arising from Chronic Myeloid Leukemia with +8, i(17q)(q10) and der(22)t(9;22) After Imatinib Therapy

Peng Shan^{1,2}, Hang Dong^{1,2}, Shilan Li³

¹Department of Laboratory, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, People's Republic of China; ²Core Unit of National Clinical Research Center for Laboratory Medicine, Hefei, Anhui, 230036, People's Republic of China; ³Department of Pathology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, People's Republic of China

Correspondence: Shilan Li, Department of Pathology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, People's Republic of China, Tel +86-0551-62284147, Email shilanli980@sina.com

Abstract: Acute basophilic leukemia (ABL) arising from chronic myeloid leukemia (CML) with abundant mast cells (MCs), coexisting with a complex karyotype is rare. Here, we report an 81-year-old man admitted to our hospital with a history of ABL. He was diagnosed with CML in the chronic phase in January 2018, and Imatinib was used at a daily dose of 400mg. Then, transformation to ABL with abundant MCs in the bone marrow and complex karyotypes including 48,XY, trisomy 8 (+8), isochromosome 17(q10) [i(17)(q10)], and derivative chromosome 22 t(9;22) [der(22)t(9;22)] were discovered simultaneously in January 2022. In conclusion, the increased number of MCs in our case is a reminder that they might play an important role in the prognosis of CML and trigger the development of complex karyotypes. Moreover, this is the first case report of ABL arising from CML with abundant MCs, coexisting with 48,XY, +8, i(17)(q10), and der(22)t(9;22), during Imatinib treatment. Further studies are needed to better characterize this rare condition.

Keywords: CML, TKIs, MCs, ABL, complex karyotype

Introduction

Acute basophilic leukemia (ABL) is an uncommon form of acute myelogenous leukemia (AML), characterized by blasts with basophilic granules. Different from mast cells (MCs), basophils show toluidine blue positivity on cytochemical staining, an immunophenotype on flow cytometry with the expression of myeloid markers, such as CD13, CD33, CD34, HLA-DR and a diverse group of cytogenetic abnormalities.

Acute basophilic leukemia arising from chronic myeloid leukemia (CML) is uncommon, and the simultaneous development of complex karyotypes in the same patient, including 48,XY, +8, i(17)(q10), and der(22)t(9;22), has not been reported previously. Here, we report an 81-year-old man diagnosed with acute basophilic leukemia arising from CML. The patient was diagnosed with CML in the chronic phase in January 2018 and treated with Imatinib at a daily dose of 400mg. Under this treatment, the disease progressed four years later in January 2022. Toluidine blue positivity on cytochemical staining demonstrated there were 51% basophils in peripheral smear, of which 22% resembled basophilic precursors. Acute basophilic leukemia was suspected. Then the patient underwent bone marrow biopsy. Immunophenotype on flow cytometry and immunohistochemistry demonstrated 30% MCs in the marrow biopsy. Chromosome analysis revealed an abnormal karyotype, including 48,XY, +8, i(17)(q10), and der(22)t(9;22). No ABL-associated fusion genes, elevated serum tryptase levels, or *KIT D816V* were found. As a result, a diagnose of acute basophilic leukemia arising from CML was made.

Case Presentation

An 81-year-old man with a history of chronic myeloid leukemia (CML) was readmitted to our hospital with leukocytosis, serious anemia and thrombocytopenia. In January 2018, bone marrow fluorescence in situ hybridization (FISH) analysis revealed the *BCR::ABL1* fusion gene and cytogenetic analysis revealed t(9;22). An initial diagnosis of CML in the chronic phase was made. He was treated with Imatinib at a daily dose of 400 mg. The patient was readmitted to our hospital because of a rising white blood cell (WBC) count and increased number of circulating blasts in February 2022. Blood work-up showed marked leukocytosis ($66.48 \times 10^9/L$), anemia (7.9 g/dl), and thrombocytopenia ($94 \times 10^9/L$), and blast crisis was suspected. A peripheral blood film showed that 51% of cells had the characteristics of basophils, of which 22% resembled to basophilic precursors, with a medium-sized, round nucleus and moderately basophilic cytoplasm containing a variable number of coarse basophilic granules (Figure 1a), which were positive for toluidine blue (Figure 1b) and negative for peroxidase (Figure 1c).

Bone marrow biopsy revealed a large proportion (more than 30%) of unexpected small round cells with sparse granules (Figure 2a). The most challenging thing was the classification of these cells. Immunohistochemistry (IHC) revealed that 10% were CD34-positive myeloblasts (Figure 2b), and more than 30% of the cells were most likely a mastocytic differentiation, because they showed strong expression of CD117 (Figure 2c) and were negative for CD25. Immunophenotyping up on flow cytometric study in bone marrow aspiration demonstrated that the neoplastic cells were subsequently revealed to exhibit a bi-differentiation. In which group one accounting for 13.8%, expressing CD13 and CD33 (Figure 3a) resemble to basophilic precursors; and group two accounting for 28.8%, strongly expressing CD117 and CD25 (Figure 3b), demonstrating they were MCs lineage.

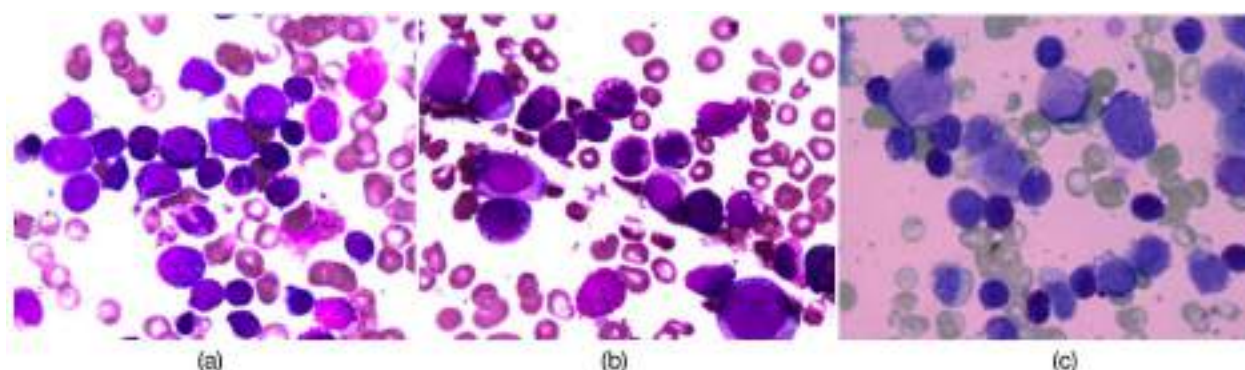


Figure 1 Morphology and cytochemistry of peripheral and bone marrow smear of tumor cells. Basophilic precursors with medium-size, round nucleus and moderately basophilic cytoplasm containing a variable number of coarse basophilic granules (a). Marrow smear showed these atypical basophils were positive for toluidine blue (b) and negative for peroxidase (c).

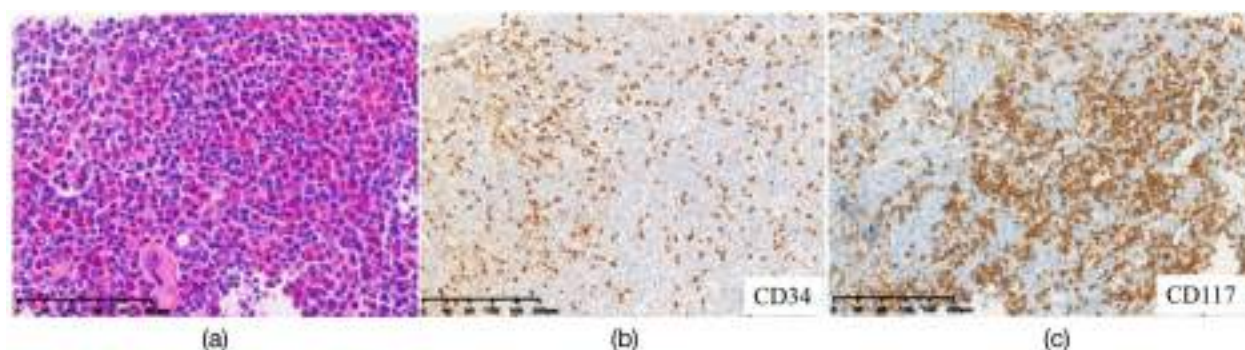


Figure 2 Morphology and immunohistochemistry of bone marrow biopsy. Biopsy showed a hypercellularity BM. The tumor cells shared similar histology morphology characteristics with the myeloid blast cells. And a large proportion of unexpected small round cells with sparse basophilic granules was discovered (a). Myeloblast showed positive for CD34 (b). Immunostaining revealed there were more than 30% of cells strongly positive for CD117 (c).

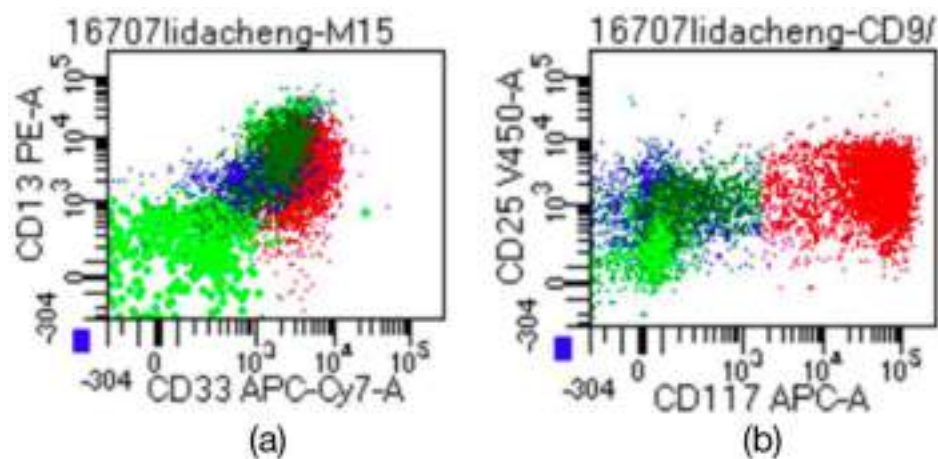


Figure 3 Immunophenotyping up on flow cytometric study in bone marrow aspiration demonstrated that the neoplastic cells were subsequently revealed to exhibit a bi-differentiation. Basophilic precursors accounting for 13.8%, expressing CD13 and CD33 (a); and mast cells accounting for 28.8%, strongly expressing CD117 and CD25 (b).

However, lymphadenopathy, cutaneous involvement, signs of hyperhistaminemia, or *KIT D816V* mutation were not detected.

FISH analysis confirmed *BCR::ABL* fusion, and cytogenetic analysis revealed complex karyotypes: 48,XY, +8, i(17)(q10), and der(22)t(9;22). All these changes were discovered after the transformation of CML to ABL. It is unknown if these abnormalities arose secondary to the Imatinib therapy, or if they accelerated the blast transformation of CML presenting with basophilic differentiation. Further studies are needed to better characterize this rare condition.

As a result, a diagnosis of acute basophilic leukemia secondary to CML in blast crisis with a complex karyotype coexisting with abundant MCs was made. After this diagnosis, the patient was treated with Dasatinib at a daily dose of 200mg. At the time of writing, the patient was well, and his WBC count had decreased to $5.16 \times 10^9/L$.

Discussion

Acute basophilic leukemia (ABL) is a rare form of acute leukemia, first described by Joachim in 1906.¹ It was recognized as a distinct entity by the most recent World Health Organization classification of myeloid malignancies. However, to date, no generally accepted criteria have been agreed for the diagnosis and classification of ABL. Acute basophilic leukemia cases secondary to chronic myeloid leukemia (CML) are rare and often represent blast transformation.² In some patients, basophilia may be excessive and may even produce a clinical picture resembling (secondary) to basophilic leukemia.

Morphologically, basophilic blasts are typically characterized by a high nucleus-to-cytoplasm ratio, round to indented nuclei with an open chromatin pattern, and abundant basophilic granules. Cytochemical staining shows a unique pattern of metachromatic positivity with toluidine blue or Giemsa, but are negative with myeloperoxidase. Different from basophilic blasts, basophils expressing myeloid markers, including CD13, CD33, CD34, and HLA-DR.

Mast cells (MCs) exert immunosuppression and stimulate immune tolerance and tumor promotion. Xu et al found that the number of MCs in CML patients increased significantly compared with the healthy group. The increasing number of MCs in the bone marrow along with the development of CML may herald the transformation of CML into AML. The number of MCs may have an important inverse relationship with the prognosis of CML. They might also act as a new therapeutic target for the treatment of tumors.³ In our present case, the bone marrow biopsy showed more than 30% MCs, which is an increase in the number of MCs compared with the patient's bone marrow biopsy from 2018. This suggests that MCs might participate in tumor development.

In our present case, the most challenging thing was differentiating basophils and MCs because of their similar morphological, phenotypic and functional characteristics. Basophils and tissue MCs represent two distinct lineages within the hematopoietic cell family. In contrast to MCs, basophils usually develop and complete their differentiation in

the bone marrow (BM) and are released into the peripheral blood (PB) after maturation. Toluidine blue activity can be observed in basophils, while the same reaction is negative in MCs.⁴ Mature basophils are positive for CD25 and negative for CD117. Diffuse and confluent CD117 positivity and CD25 negativity in our case demonstrated that the cells were mature MCs.

Ninety percent of patients have a typical t(9;22), resulted in the formation of the fusion gene *BCR::ABL1*; while 5% have variant translocations, which can be simple (involving chromosome 9 and a chromosome other than chromosome 22), or complex (involving one or more chromosomes in addition to chromosomes 9 and 22). Additional chromosomal abnormalities are reported in 5%–10% of cases, including double Ph, trisomy 8, isometric 8, isochromosome 17, and isometries of chromosomes 19 and 20.^{5,6} Our case was first diagnosed with CML in the chronic phase in January 2018 and was treated with Imatinib at a daily dose of 400mg. He visited our hospital in February 2022, when bone marrow biopsy demonstrated progression to ABL, and chromosome genetic analysis revealed a complex karyotype 48,XY, +8, i(17)(q10), and der(22)t(9; 22). ABL- associated fusion genes, elevated serum tryptase levels and *KIT D816V* mutation were not found.

Because of the patient's poor clinical status and drug resistance, he was treated with Dasatinib, and the percentage of *BCR::ABL1* positive cells decreased to 2.16% after eight months of treatment.

The therapeutic scheme currently used involves tyrosine kinase inhibitors (TKIs) associated with high-intensity systemic chemotherapy. Imatinib has long been the first-choice drug for CML therapy because of durable responses and prolonged patient survival. Unfortunately, clinical complications can occur during treatment, in particular the onset of resistance due to point mutation of *BCR::ABL1*.

Dasatinib is used to treat Imatinib resistant patients with *BCR::ABL1* point mutations, except in the presence of T315I mutation.⁷ We suspect these changes might result from somatic mutation of blast cells during TKI therapy. It is unknown whether the complex karyotype and the presentation of basophilic precursors in this CML blast crisis were related to the Imatinib therapy, or if these changes accelerated this patient into blast crisis. There are no guidelines on treatment in this scenario because of the rarity of this presentation, and it is assumed that patients with this clinical presentation have a high rate of mortality after diagnosis. Further study is therefore needed to better characterize this rare condition, investigate prognostic factors, and define optimal management.

Conclusion

The treatment regimen for acute basophilic leukemia secondary to CML still present a great challenge for clinicians, especially when accompanied by complex karyotypes. The increasing number of MCs in our case is a reminder that they might play an important role in the prognosis of CML. Further studies are needed to better characterize this rare condition.

Data Sharing Statement

All authors have declared the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study complies with all relevant ethical regulations and the study protocol was approved by the Biomedical Ethics Committee of USTC (2022-RE-335). The specimens of paraffin sections used in this study were obtained in previous clinical diagnosis and treatment, which will not cause physical and mental suffering to the patient. Written informed consent has been provided by the patient to have the case details, including photographs, case history and details published. The privacy and personal information of the patient will be protected, and the specimens that subjects have explicitly refused to use will not be used.

Acknowledgments

We are grateful to Dr Hang Dong for the diagnosis of peripheral blood and bone marrow smear. We also thank the department of Flow Cytometry and the central laboratory of the First Affiliated Hospital of USTC for their work in the diagnosis of this case.

Author Contributions

We thank Peng Shan and Shilan Li for made a significant contribution to the work reported. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. All the authors declare that there is no conflict of interest.

References

1. Ber JGU. Mastzellenleukamien. *Dtsch Arch Klin Med*. 1906;87:437.
2. Gupta R, Jain P, Anand M. Acute basophilic leukemia: case report. *Am J Hematol*. 2004;76(2):134–138. doi:10.1002/ajh.10446
3. Xu P, Zhang C, Wang Y, et al. Increased number of mast cells in the bone marrow of chronic myeloid leukemia may herald the pending myeloid transformation—the mast cell is an indicator of myeloid transformation. *Transl Cancer Res*. 2019;8(5):2121–2129. doi:10.21037/tcr.2019.09.29
4. Peterson LC, Parkin JL, Arthur DC, Brunning RD. Acute basophilic leukemia. A clinical, morphologic, and cytogenetic study of eight cases. *Am J Clin Pathol*. 1991;96(2):160–170. doi:10.1093/ajcp/96.2.160
5. Rojas-Atencio A, Urdaneta K, Soto-Quintana M, Alvarez Nava F, Canizales J, Solis E. Trisomy 19 and t(9;22) in a patient with acute basophilic leukemia. *Case Rep Pathol*. 2011;2011:269491. doi:10.1155/2011/269491
6. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2022 update on diagnosis, therapy, and monitoring. *Am J Hematol*. 2022;97(9):1236–1256. doi:10.1002/ajh.26642
7. Di Maria S, Picarazzi F, Mori M, et al. Novel pyrazolo[3,4-d]pyrimidines as dual Src/Bcr-Abl kinase inhibitors: synthesis and biological evaluation for chronic myeloid leukemia treatment. *Bioorg Chem*. 2022;128:106071. doi:10.1016/j.bioorg.2022.106071

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Flow Cytometric Enumeration of Peripheral Blood CD34⁺ Cells Predicts Bone Marrow Pathology in Patients with Less Than 1% Blasts by Manual Count

Tomislav M Jelic, Oscar C Estalilla, Jeffrey A Vos, Gary Harvey, Caitlin J Stricker, Ayodele O Adelanwa, Ahmed A Khalid & Milton J Plata

To cite this article: Tomislav M Jelic, Oscar C Estalilla, Jeffrey A Vos, Gary Harvey, Caitlin J Stricker, Ayodele O Adelanwa, Ahmed A Khalid & Milton J Plata (2023) Flow Cytometric Enumeration of Peripheral Blood CD34⁺ Cells Predicts Bone Marrow Pathology in Patients with Less Than 1% Blasts by Manual Count, Journal of Blood Medicine, , 519-535, DOI: [10.2147/JBM.S417432](https://doi.org/10.2147/JBM.S417432)

To link to this article: <https://doi.org/10.2147/JBM.S417432>



© 2023 Jelic et al.



Published online: 21 Sep 2023.



[Submit your article to this journal](#)



Article views: 262



[View related articles](#)



[View Crossmark data](#)



Citing articles: 3 [View citing articles](#)

Flow Cytometric Enumeration of Peripheral Blood CD34⁺ Cells Predicts Bone Marrow Pathology in Patients with Less Than 1% Blasts by Manual Count

Tomislav M Jelic¹, Oscar C Estalilla¹, Jeffrey A Vos², Gary Harvey¹, Caitlin J Stricker¹, Ayodele O Adelanwa², Ahmed A Khalid³, Milton J Plata¹

¹Department of Pathology and Laboratory Medicine, Charleston Area Medical Center, Charleston, WV, USA; ²Department of Pathology, Anatomy and Laboratory Medicine, West Virginia University School of Medicine, Morgantown, WV, USA; ³Cancer Center, Charleston Area Medical Center, Charleston, WV, USA

Correspondence: Tomislav M Jelic, Charleston Area Medical Center, Department of Pathology and Laboratory Medicine, 3200 MacCorkle Avenue, Charleston, WV, 25304, USA, Tel +1 304 546 6714, Email tomilsavjelic@suddenlink.net

Background and Aims: Significance of absolute number of CD34⁺ cells in the peripheral blood of patients with less than 1% myeloblasts by manual differential count is unknown and our aim is to study its relevance in clinical practice.

Methods: We studied 138 peripheral bloods flow cytometric analyses in patients with less than 1% myeloblasts by manual differential, when CD34⁺ events were present in the gate that encompassed lymphocytes, monocytes, stem cells, and blasts.

Results: The average absolute number of CD34⁺ cells in the peripheral blood was 11 CD34⁺ cells/ μ L ranging from less than 1 cell/ μ L to 147 cells/ μ L. The average absolute number of CD34⁺ cells in patients with an abnormal expansive process involving bone marrow (metastases, myelodysplasia, granulomas, marrow infections) or if bone marrow biopsy not performed, presumed expansive marrow process was 25 cells/ μ L, and in patients without an expansive marrow process (or presumed negative) was 4 cells/ μ L ($P < 0.00007$). Cutoff 12 CD34⁺ cells/ μ L had 93% positive predictive value for bone marrow involvement by an expansive process and 78% negative predictive value.

Conclusion: Flow cytometric testing of the peripheral blood is extremely sensitive method for enumerating CD34⁺ cells and can detect fewer than one CD34⁺ cell/ μ L. The absolute number of CD34⁺ cells in the peripheral blood is a useful parameter in determining marrow involvement by an expansive process and may provide guidance with respect to the necessity for bone marrow biopsy.

Keywords: flow cytometry, CD34 positive cells, peripheral blood, myeloblasts, bone marrow biopsy

Introduction

Although numerous advances in characterization of myeloid neoplasms largely derived from gene expression analysis and next generation sequencing are now available, the percentage of myeloblasts in the bone marrow and peripheral blood remains the most important parameter in diagnosing acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms.¹ When myeloblasts are detected by microscopic examination of the peripheral blood smear, a subsequent bone marrow study is typically necessary to determine the significance of this finding. Microscopic examination of the peripheral blood with a differential count of 100 white blood cells, as a method of detecting blasts, is of limited accuracy and sensitivity. For example, one percent myeloblasts in a patient with a white blood cell count of 7,000 WBC/ μ L (white blood cells/microliter of blood) indicates that there are already 70 myeloblasts/ μ L in the peripheral blood. The sensitivity at such low percentages may only be improved upon by counting increasing numbers of cells. Flow cytometric analysis of peripheral blood usually counts 20,000 leukocytes (ie, events) and is thus about 200 times more sensitive than manual counting for detecting the low-level presence of myeloblasts in the peripheral blood. Enumeration of the absolute number of CD34⁺ cells in the peripheral blood by flow cytometry has been performed for more than 28 years.² Basal levels of CD34⁺ cells in peripheral blood differ between individuals and are typically stable

for 18 months.³ The CD34⁺ cells, which most frequently represent myeloblasts co-expressing CD34 and CD33, are present in the circulation in about one-tenth the concentration seen in the bone marrow (0.2% vs 1.8%).⁴ According to the medical literature^{3,5} and our study, flow cytometric analysis of the peripheral blood can accurately detect and enumerate even less than one myeloblast/ μ L.

At the time of this study, there are no reports in the medical literature concerning the absolute number of CD34⁺ cells in the peripheral blood in patients with various peripheral blood hematological abnormalities and with less than 1% myeloblasts obtained by manual count on glass slide smear. Our study determined the significance of enumerating these low-level myeloblast percentages in the peripheral blood by comparing them to the bone marrow pathology. We found that the absolute number of CD34⁺ cells (as well as percentage) determined from these flow cytometry studies of peripheral blood, may provide a reasonable guide in determining the need for bone marrow biopsy.

Materials and Methods

Flow Cytometry

We analyzed 138 peripheral blood flow cytometric studies (133 patients, 62 women and 71 men) in whom CD34⁺ cells were recorded in a mononuclear gate that encompassed lymphocytes, monocytes, stem cells, and blasts on right angle scatter/forward scatter dot plot. Institutional Review Board approval for this study was obtained. Inclusion criterium was the presence of CD34⁺ events (cells) forming distinct cloud or cluster of bright dots comprising at least 0.1% of events on CD34/CD19 dot plot in a mononuclear gate. Patients in whom such CD34⁺ events were not detected were excluded from the study.

The lowest percentage of CD34⁺ cells in the targeted gate that our Beckman Coulter Navios flow cytometer was able to detect was 0.1%. Peripheral blood for flow cytometric study was collected in citric acid medium. Forward-light scatter versus side scatter (FSC vs SSC) gating strategy was used to select region that encompasses all mononuclear leukocytes (Figure 1) including stem cells, lymphocytes, blasts, and monocytes.⁶ This gate ensured that blasts and other progenitor cells that vary in size from small lymphocytes to monocytes would be included and that nonviable cells and residual nonlysed erythrocytes will be excluded. The absolute number of CD34⁺ cells in the peripheral blood was then obtained by using the same calculation sequence (formula) as is typically employed to determine the absolute number of T-lymphocytes in human immunodeficiency virus (HIV) infected patients.⁷ Multiparameter (six-color) flow cytometric analysis of the peripheral blood was carried out on the Beckman Coulter Navios flow cytometer. The CD34 antibody was conjugated with the allophycocyanin (APC) fluorochrome and CD34 positive events were regarded as real only when they formed a cloud or cluster of dots in the mononuclear gate and if their percentage was at least 0.1% (Figure 1). Scattered CD34⁺ dots were regarded as nonspecific staining (dust-like

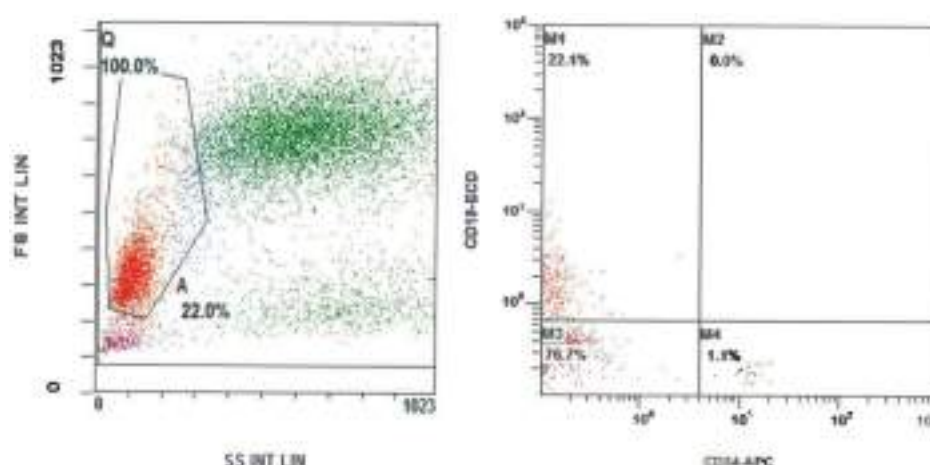


Figure 1 Flow cytometric peripheral blood forward-angle light scatter (FS)/right-angle light scatter (SS) dot plot and CD19/CD34 dot plot. Gate A (area inside black line in a trapezoid form) encompasses lymphocytes, stem cells, blasts and monocytes and excludes dead cells and nonspecific particles.

Abbreviations: FS, forward scatter; SS, side scatter; ECD, PE-Texas red tandem; APC, allophycocyanin.

artifacts) and such finding was regarded as negative for the presence of CD34⁺ cells and excluded from the study. The CD1a⁺ (coupled with APC fluorochrome) events in the targeted gate were also examined in all patients to further exclude artifacts of nonmyeloblast/stem cell events. All patients included in our study were required to show essentially no CD1a⁺ co-expressing events. This selection process was used, rather than attempting to subtract CD1a⁺ cells from CD34⁺ cells, to prevent the introduction of false positive data. Patients with T-lymphoblastic leukemia (T-ALL) were excluded from our study because they all had more than 1% blasts by manual count on the peripheral blood smear. Patients with other types of acute leukemia were also excluded because they all had at least 1% of blasts in the peripheral blood at the time when flow cytometric analysis of the peripheral blood was performed. Our panel for flow cytometric study of the peripheral blood used markers with fluorochromes as follows: Tube 1: Isotype IgG1 (FITC fluorochrome), Isotype IgG1 (Pe fluorochrome), isotype IgG1 (ECD fluorochrome), isotype IgG1 (Pc5.5 fluorochrome), CD45 (Pc7 fluorochrome), isotype IgG1 (APC fluorochrome); Tube 2: CD5 (FITC fluorochrome), CD10 (Pe fluorochrome), CD19 (ECD fluorochrome), CD20 (Pc5.5 fluorochrome), CD45 (Pc7 fluorochrome), CD22 (APC fluorochrome); Tube 3: CD Kappa (FITC fluorochrome), CD Lambda (Pe fluorochrome), CD19 (ECD fluorochrome), CD20 (Pc 5.5 fluorochrome), CD45 (Pc7 fluorochrome), CD34 (APC fluorochrome); Tube 4: CD33 (FITC fluorochrome), CD10 (Pe fluorochrome), CD13 (ECD fluorochrome), CD117 (Pc5.5 fluorochrome), CD45 (Pc7 fluorochrome), CD34 (APC fluorochrome); Tube 5: CD7 (FITC fluorochrome), CD15 (Pe fluorochrome), CD14 (ECD fluorochrome), CD HLA-DR (Pc 5.5 fluorochrome), CD45 (Pc7 fluorochrome), CD1a (APC fluorochrome), Tube 6: CD8 (FITC fluorochrome), CD4 (Pe fluorochrome), CD2 (ECD fluorochrome), CD56 (Pc5.5 fluorochrome), CD45 (Pc7 fluorochrome), CD3 (APC fluorochrome). All of these antibody (CD) markers are ASRs (analyte specific reagents) manufactured by Beckman Coulter. Analysis was performed by Beckman Coulter Navios software.

Peripheral Smear Evaluation and Complete Blood Count (CBC) Analysis

Peripheral blood smears were stained by standard Wright/Giemsa stain. A manual differential count on 100 WBC was performed by either of two hematopathologists (OCE, TMJ) with the Olympus microscope at 600× magnification. The percentage of cells was compared to that obtained by automated hematology analyzer (Beckman Coulter DxH 900) and if discrepancy was present, recounting was performed.

Whole blood was collected in EDTA medium and CBC analysis was performed by automated hematology analyzer (Beckman Coulter DxH 900). The leukocyte count obtained from this analysis was subsequently used in the calculation of circulating CD34⁺ cells.

Enumeration of CD34⁺ Cells

The absolute number of CD34⁺ cells was calculated by utilizing a dual platform-based method consisting of data obtained from the flow cytometry study, number of leukocytes in the peripheral blood determined by CBC analysis, and sum of the percentage of lymphocytes and monocytes obtained by a manual differential count. The calculation method is presented here first by mathematical equation and then in detail step by step. Absolute number of CD34⁺ cells = (absolute number of mononuclear cells) × (percentage of CD34⁺ cells in mononuclear gate)/100.

For patient number (#) 42 in Table 1, Figure 1, a 62-year-old-man with presumed marrow involvement by mantle cell lymphoma, the absolute number of CD34⁺ cells in the peripheral blood was obtained as follows: a manual differential count yielded: 69% neutrophils, 2% bands, 2% eosinophils, 1% LGL (large granular lymphocytes), 20% small lymphocytes, and 6% monocytes. The sum of mononuclear cells (percentage of lymphocytes + percentage of monocytes) was 27% and with 13,600 WBC/ μ L, it yielded 3672 (lymphocytes + monocytes)/ μ L. Flow cytometric study showed that in the lymphocytic-monocytic gate (mononuclear gate) the percentage of CD34⁺ cells was 1.1% (Figure 1). This means that 1.1% of 3672 is the absolute number of CD34⁺ cells in the peripheral blood or 40.392 cells/ μ L ($1.1 \times 3672/100$) or 40 CD34⁺ cells/ μ L. The percentage of CD34⁺ cells in the peripheral blood is 0.297% of WBC ($40.392 \times 100/13,600$).

The comprehensive clinical and laboratory data of 138 studies are presented in Table 1 and Table 2. Table 1 lists data from 48 patients (49 studies) with bone marrow involvement or presumed involvement by an expansive process (non-reactive), while Table 2 lists data from 85 patients (89 studies) in which marrow is not or supposedly not involved by an expansive process.

Table I Patients with Bone Marrow Involvement or Presumed Involvement by an Expansive Process (Excluding Simple Hyperplasia)

#	G	Age	Diagnosis	WBC	CD34 ⁺	% CD34 ⁺	BM	% mbl BM	% Lymphs + Monos	% CD34 ⁺ Cells In Ly/Mono Gate
1	w	28	Anaplastic large cell lymphoma. Splenomegaly Hgb 8.5 Trc 117	3.1	1	0.031	Y	0	31	0.1
2	w	59	Non-Hodgkin lymphoma B cells comprise 20% of marrow cells. Hgb12.3 Trc 190	4.3	2	0.042	Y	0.5	42	0.1
3	w	81	B-CLL 70–75% of marrow is occupied by B cells. Trisomy 12 Hgb 10.7 Trc 144 3 copies of MDM gene	4.8	2	0.042	Y	0.5	42	0.1
4	m	67	Low grade B cell lymphoma small B- cells occupy 25–30% of marrow, FISH-negative, MYD 88-wild, 46XY, Pancytopenia. Hgb 12.1 Trc 37	3.2	2	0.06	Y	0	60	0.1
5	w	75	RAEB-2 Monosomy 7 Hgb 10.2 Trc 121	1.8	3	0.188	ND		47	0.4
6	m	79	B-CLL occupies 95% of marrow, Hgb 7.1 Trc 46	5.3	3	0.061	Y	NA	61	0.1
7	m	83	Plasma cell dyscrasia, 10% plasma cells, 1% monoclonal B cells Hgb 10.8 Ptitl 204	5.0	3	0.056	Y	0	56	0.1
8	m	65	Multilineage dysplasia (MDS-MLD), 46XY, FISH for MDS is negative, Hgb 11.1 Trc 66 T cell receptor gene rearranged of unknown significance in bone marrow and blood. 527 LGL/μL	3.4	3	0.092	Y	0.5	46	0.2
9	w	67	Pancytopenia Hgb 9.7 Trc55360neutrohils/uL MDS-EB-I	1.8	4	0.234	Y	8.5	78	0.3
10	w	77	Marrow replaced by B cell lymphoma Hgb 14.9 Trc 274	9.7	4	0.046	Y	NA	23	0.2
11	w	64	MDS-low grade Hgb 9.8 Trc 275, 46XX, FISH-negative	1.6	4	0.249	Y	0.3	78	0.2
12	w	76	RAEB-2, Monosomy 7 Hgb 11.5 Trc 189	2.8	5	0.18	ND		36	0.5
13	w	51	Chronic phase CML. Hgb 15.6 Trc 298	14.5	5	0.036	Y	2	36	0.1
14	m	86	Pancytopenia Hgb 10.0 Trc 108	1.1	5	0.441	ND		63	0.7
15	w	68	MDS-MLD. Point mutation-NGS. Hgb 11.1 Trc 88	4.4	6	0.142	Y	0	71	0.2
16	w	71	Pancytopenia, Sarcoidosis H Hgb 11.6 Trc 14	4.6	6	0.129	ND		43	0.3
17	m	61	Prostate cancer MCV 114.3 fL Hgb 15.0 Trc 250	12.5	7	0.058	ND		29	0.2
18	m	71	In PB 3200 B-CLL cells/μL. Hgb 14.6 Trc 258 Merkel cell carc	12.8	7	0.058	ND		58	0.1

19	w	59	2725 B-CLL cells/ μ L Hgb 14.1 Trc 260	13.2	8	0.059	ND		59	0.1
20	m	56	Hgb 12.5 TrcI 44. Two years later in marrow plasma cell dyscrasia, and lymphoma not specified.	12.5	8	0.06	Y	NA	30	0.2
21	m	53	5888 B-CLL cells/ μ L Hgb 16.2 Trc 214	18.4	10	0.052	ND		52	0.1
22	m	66	1540 LGL/ μ L Hgb 15.6 Trc 190 T cell receptor gene is rearranged	7.0	10	0.142	ND		71	0.2
23	m	95	Bicytopenia Hgb 11.2 Trc 262	2.7	10	0.378	ND		21	1.8
24	w	90	3650 B-CLL cells/ μ L Hgb 9.3 Trc 210	8.3	11	0.132	ND		66	0.2
25	m	80	8160 B lymphoma cells/ μ L Hgb 14.2 Trc 167	17.0	12	0.071	ND		71	0.1
26	w	79	MDS-MLD complex unfavorable karyotype Hgb 9.5 Trc 235	5.7	13	0.231	Y	1.2	33	0.7
27	m	57	8152 B-CLL cells/ μ L Hgb 15.9 Trc 169. 3 years ago, renal cell carcinoma	19.4	14	0.072	ND		72	0.1
28	m	51	7412 B-CLL cells/ μ L Hgb 15.5 Trc 282	21.8	15	0.069	ND		71	0.1
29	m	62	7456 B-CLL cells/ μ L Hgb 16.2 Trc 115	23.3	16	0.069	ND		69	0.1
30	w	67	Classical Hodgkin lymphoma mixed cellularity Hgb 8.6 Trc 278	26.0	17	0.065	ND		13	0.5
31	w	48	MDS-MLD Hgb 7.3 Trc 28	1.9	17	0.91	Y	NA	70	1.3
32	m	63	Prostate cancer Grade group I Hgb 15.6 Trc 248	16.5	18	0.108	ND		54	0.2
33	m	70	Small cell lung cancer. Hgb 13.4 Trc 26	16.5	22	0.133	ND		19	0.7
34	m	71	Adenocarcinoma replacing marrow. Hgb 6.6 Trc 37	8.5	22	0.256	Y	NA	16	1.6
35	m	76	6280 B cells/ μ L Hgb 13.9 Trc 317	20.5	23	0.114	ND		57	0.2
36	m	66	Mantle cell lymphoma involves 70% of marrow. Hgb 8.5 Trc 101	14.9	24	0.016	Y	0	80	0.2
37	w	88	17 CD10+ B cells/ μ L. Hgb 8.6 Trc 45	3.5	25	0.715	ND		55	1.3
38	m	74	CML Hgb 13.4 Trc 420	29.6	27	0.092	Y	0.3	23	0.4
39	m	73	Prostate adenocarcinoma, bone marrow metastases-autopsy. Hgb 11.0 Trc 39	17.0	35	0.204	ND		35	1.2

(Continued)

Table I (Continued).

#	G	Age	Diagnosis	WBC	CD34 ⁺	% CD34 ⁺	BM	% mbl BM	% Lymphs + Monos	% CD34 ⁺ Cells In Ly/Mono Gate
40	m	37	Chronic phase CML, Hgb 11.8 Trc 81	23.8	37	0.155	ND		31	0.5
41	m	74	<i>Cryptococcus</i> meningitis Hgb 8.5 Trc 205	23.8	38	0.16	ND		20	0.8
42	m	62	414 mantle cells/ μ L Hgb 9.3 Trc 55	13.6	40	0.297	ND		27	1.1
43	w	82	38,645 B-CLL-cells/ μ L Hgb 13.0 Trc 161	20.3	49	0.089	ND		89	0.1
44	m	64	14,515 Mantle cells/ μ L Hgb 7.0 Trc 77	20.3	52	0.258	ND		86	0.3
45	m	72	65,333 B-CLL cells/ μ L Hgb 7.7 Trc 15	82.7	80	0.097	Y	NA	97	0.1
46	m	80	91,140 B-CLL cells/ μ L Hgb 8.4 Trc 153	13.02	109	0.084	ND		84	0.1
47	m	72	Primary myelofibrosis, Hgb 8.3 Trc 342	50	115	0.23	ND	NA	10	2.3
48	m	88	41,186 B-CLL cells/ μ L Hgb 8.5 Trc 46	65.0	126	0.194	ND		97	0.2
49	m	83	Primary myelofibrosis, JAK-2 mutation+ Hgb 12.4 Trc 735	20.8	147	0.705	Y	0	15	4.7

Note: #=number.

Abbreviations: G, gender; w, woman; m, man; Hgb, hemoglobin (g/dL); Trc, thrombocytes ($10^3/\mu$ L); FISH, fluorescence in situ hybridization; i.v., intravenous; MDS, myelodysplastic syndrome; MLD, multilineage dysplasia; B-CLL-B-cell chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; WBC, white blood count $10^3/\mu$ L; BM, bone marrow; Y, Yes; ND, not done; NA, not applicable (bone marrow smear or touch preparation of insufficient quality to do differential count); Lymphs, lymphocytes; Monos, monocytes; Ly/Mono, lymphocytic/monocytic.

Table 2 Patients with No Bone Marrow Involvement or Presumed Uninvolvement by an Expansive Process (Excluding Simple Hyperplasia)

#	G	Age	Diagnosis	WBC	CD34+	%CD34+	BM	% mbl BM	% Lymphs + Monos	% CD34+ Cells in Ly/Mono Gate
1	w	60	Pancytopenia Hgb 10.3 Trc 36	1.8	0.38	0.021	ND		21	0.1
2	w	26	Pancytopenia, i.v. drug abuse, acute endocarditis, HIV, hepatitis C. Hgb 10.1 Trc 11	0.5	0.38	0.076	ND		76	0.1
3	m	59	Pancytopenia, 5-years ago stem cell transplantation for ALL. Hgb 9.0 Trc 139	4.6	0.46	0.01	ND		10	0.1
4	w	65	Bicytopenia, pseudthrombocytopenia, 205 T4-cells/ μ L Hgb 9.4 Trc 40	2.5	0.77	0.031	ND		31	0.1
5	w	71	Pancytopenia. Hgb 8.1 Trc 82	2.8	0.84	0.03	ND		15	0.2
6	W	39	i.v. drug abuser, Chronic hepatitis C. Microcytic anemia, Hgb 10.0 Trc 270	5.5	0.88	0.016	ND		16	0.1
7	w	72	Pancytopenia due to methotrexate for acute kidney injury. Hgb 7.9 Trc 46	0.6	0.9	0.156	ND		78	0.2
8	m	72	Anemia of chronic inflammation Hgb 10.2 Trc 176	3.0	0.93	0.031	ND		31	0.1
9	w	37	Pancytopenia, alcoholic liver cirrhosis. Hgb 9.1 Trc 80 platelet clumps	4.7	1	0.025	ND		25	0.1
10	w	71	Mild leukopenia Hgb 14.2 Trc 168	4.8	1	0.032	ND		32	0.1
11	w	33	Autoimmune hemolytic anemia Hgb 9.8 Trc 224	4.5	1	0.027	ND		27	0.1
12	w	78	Cold hemagglutinin, 1248 neutrophils/ μ L	2.6	1	0.049	ND		49	0.1
13	m	38	Pancytopenia Hgb 9.6 Trc 113	1.2	1	0.122	ND		61	0.2
14	w	46	Autoimmune hemolytic anemia Hgb 13.9 Trc 254	10.7	2	0.017	ND		17	0.1
15	w	29	Easy bruising Hgb 13.2 Trc 361	8.2	2	0.03	ND		30	0.1
16	m	56	Coombs positive hemolysis Hgb 14.5 Trc 356	4.5	2	0.054	ND		54	0.1
17	w	47	Mild thrombocytosis Hgb 13.0 Trc 458	5.4	2	0.033	ND		33	0.1
18	m	70	Nephrectomy for renal cell carcinoma 3 years ago. Hgb 14.4 Trc 140	7.4	2	0.029	ND		29	0.1
19	w	58	Colecotomy for ulcerous colitis, anemia. Leukopenia Hgb 11.0 Trc 248	3.9	2	0.051	ND		51	0.1
20	m	58	Prostate cancer group Grade 1, acute myocardial infarction Hgb 13.3 Trc 176	11.3	2	0.014	ND		7	0.2
21	w	36	Leukopenia Hgb 13.2 Trc 194	4.2	2	0.047	ND		47	0.1

(Continued)

Table 2 (Continued).

#	G	Age	Diagnosis	WBC	CD34+	%CD34+	BM	% mbl BM	% Lymphs + Monos	% CD34+ Cells in Ly/Mono Gate
22	w	63	Hgb 14.7 Trc 106. Mildly enlarged abdominal lymph nodes	8.9	2	0.025	ND		25	0.1
23	w	81	Serum monoclonal spike 0.2 g/dl, mild anemia Hgb 11.0 Trc 275	6.4	2	0.027	ND		27	0.1
24	w	50	HIV+ recurrent shingles Hgb 13.2 Trc 263	7.7	2	0.031	ND		31	0.1
25	m	33	Chronic renal failure on dialysis. Pancytopenia Hgb 9.1 Trc 93	2.8	2	0.069	ND		23	0.3
26	m	66	Persistent leukopenia Hgb 15.7 Trc 217, 1365 neutrophils/ μ L	3.5	2	0.06	ND		60	0.1
27	m	49	Mild leukocytosis Hgb 14.7 Trc 223	9.9	2	0.025	ND		25	0.1
28	m	53	Follicular lymphoma grade 2 treated. 2 years ago Hgb 15.3 Trc 202	6.2	2	0.029	ND		29	0.1
29	w	60	Iron deficiency anemia, Nodular sclerosis Hodgkin lymphoma, blastomycosis, Hgb 8.7 Trc 627	11.7	2	0.016	ND		16	0.1
30	m	75	Mild thrombocytopenia Hgb 14.2 Trc 134	9.1	2	0.022	ND		22	0.1
31	m	60	Follicular lymphoma grade 2. Bone marrow is not involved Hgb 16.5 Trc 226	5.9	2	0.038	Y	1.5	38	0.1
32	w	71	Long time neutropenia likely due to drugs. 2016 neutrophils/ μ L Hgb 12.8 Trc 218	3.2	2	0.066	ND		33	0.1
33	w	68	Leukocytosis due to asthma treatment with corticosteroids. Hgb 9.5 Trc 423	18.0	2	0.012	ND		6	0.2
34	m	23	Hepatosplenomegaly, trace ascites. Hgb 15.0 Trc 321	7.6	2	0.026	ND		16	0.1
35	w	68	Systemic lupus erythematosus. Hgb 11.9 Trc 224	4.9	2	0.051	ND		51	0.1
36	m	69	Pancytopenia Hgb 7.0 Trc 90	3.2	2	0.062	ND		31	0.2
37	m	66	Leukopenia Hgb 15.7 Trc 142	3.9	2	0.048	ND		48	0.1
38	m	86	Pancytopenia Hgb 13.1 Trc 135	1.4	2	0.162	Y	0	81	0.2
39	w	45	iv. drug abuser, hepatitis. Hgb 13.0 Trc 89	3.0	2	0.052	ND		52	0.1
40	w	55	Subacute combine degeneration, epilepsy, Hgb 11.1 MCV 104.2 fL, Trc 380	4.7	2	0.048	ND		48	0.1

41	m	77	Anemia, eosinophilia 29%, Hgb 10.7 Trc 319	15.4	3	0.022	ND		11	0.2
42	m	71	Mycosis fungoides, Hgb 16.2 Trc 291	8.03	3	0.035	Y	I	35	0.1
43	m	79	Primary skin marginal cell lymphoma, Hgb 14.8 Trc 192	6.5	3	0.042			42	0.1
44	w	57	Necrotizing granulomas in lymph nodes. Hgb 12.4 Trc 384	6.0	3	0.052	ND		52	0.1
45	m	30	Liver cirrhosis, Hgb 7.3 Trc 90	7.2	3	0.044	Y	NA	11	0.4
46	w	26	Hidradenitis suppurativa Hgb 13.2 Trc 368	8.1	3	0.042	ND		42	0.1
47	w	26	Hidradenitis suppurativa Hgb 15.0 Trc	6.7	3	0.04	ND		40	0.1
48	m	78	Possible cryoglobulin, Hgb 13.9 Trc 302	13.4	3	0.025	ND		25	0.1
49	m	68	Pancytopenia Hgb 12.3 Trc 34	4.4	3	0.066	ND		22	0.3
50	w	84	Deep vein thrombosis, pulmonary embolus, Hgb 12.2 Trc 289	6.3	3	0.042	ND		42	0.1
51	w	29	2,104 neutrophils/ μ L 477 eosinophils/ μ L, Hgb 13.0 Trc 219	5.3	3	0.051	ND		51	0.1
52	w	85	1250 neutrophils/ μ L, Hgb 8.2 Trc 103	5.0	3	0.055	ND		55	0.1
53	w	52	575 LGL/ μ L Hgb 13.7 Trc 331	11.5	4	0.039	ND		39	0.1
54	m	60	507 polyclonal B cells/ μ L Hgb 16.4 Trc 191	8.0	4	0.047	ND		47	0.1
55	w	65	Hgb 15.0 Trc 185 No other data available	7.2	4	0.052	ND		52	0.1
56	w	65	Hgb 12.4 MCV 76.7 fL, Trc 401, 49% lymphocytes, 7% monocytes	7.2	4	0.056	ND		56	0.1
57	w	67	58% lymphocytes, Hgb 13.0 Trc 336	6.9	4	0.063	ND		63	0.1
58	m	60	B cell small lymphocytic lymphoma, 179 CD5+CD19+ B cells/ μ L Hgb 14.8 Trc 292	5.1	4	0.072	ND		36	0.2
59	w	48	Diabetic neuropathy, Hgb 13.1. Trc 307	9.8	4	0.037	ND		37	0.1
60	m	22	2,058 neutrophils/ μ L Hgb 18.4 Trc 158, Body builder	4.2	4	0.096	ND		48	0.2
61	w	63	Colon cancer treated by surgery, 5-FU, irradiation. Hgb 13.5 Trc 283	2.9	4	0.154	ND		77	0.2
62	w	30	4788 lymphocytes/ μ L Hgb 13.4 Trc 293	12.6	5	0.038	ND		38	0.1

(Continued)

Table 2 (Continued).

#	G	Age	Diagnosis	WBC	CD34+	%CD34+	BM	% mbl BM	% Lymphs + Monos	% CD34+ Cells in Ly/Mono Gate
63	m	74	Alcoholic fatty liver, Hgb 16.5, MCV 104.0 fL Trc 104	11.8	5	0.039	ND		39	0.1
64	w	70	Monoclonal gammopathy of unknown significance. Hgb 11.2 MCV 78.8 fL. Trc 255	10.7	5	0.048	ND		48	0.1
65	m	51	Hairy cell leukemia 13 years ago. Now 1456 LGL/μL Hgb 15.0 Trc 277	10.4	5	0.049	ND		49	0.1
66	m	72	Axillary lymph node metastasis of melanoma. Hgb 15.2 Trc 131	9.5	5	0.055	ND		55	0.1
67	w	27	Hidradenitis suppurativa. 588 LGL/μL Hgb 13.0 Trc 279	8.4	5	0.057	ND		57	0.1
68	w	66	1072 LGL/μL. T cell gene receptor rearranged, Hgb 14.8 Trc 401	7.4	5	0.064	ND		64	0.1
69	w	46	Follicular lymphoma g. 1–2. Hgb 15.1 Trc 255	7.6	5	0.069	ND		23	0.3
70	m	58	Platelet clumps Trc 141 Hgb 15.4, 452 polyclonal B cells/μL	5.3	5	0.104	ND		52	0.2
71	w	66	1554 neutrophils/μL. Hgb 14.0 Trc 352	4.2	5	0.126	ND		63	0.2
72	w	62	612 neutrophils/μL. Hgb 13.9 Trc 289, Colon cancer treated by surgery, 5-FU, irradiation	3.4	5	0.142	Y	NA	71	0.2
73	m	62	Chronic obstructive lung disease. Hgb 13.6 Trc 283	10.7	6	0.058	ND		29	0.2
74	w	56	1768 LGL/μL, 739 polyclonal B cells/μL Trc 396, 2149 T4-cells/μL. 1741 T8-cells/μL Hgb 12.9	13.6	6	0.048	ND		48	0.1
75	w	38	Seronegative rheumatoid arthritis Hgb 12.7, Trc 443	14.0	6	0.045	ND		45	0.1
76	w	66	Erythrocytes 5.79 Hgb 16.9 Trc 454. Molecular studies for myeloproliferative neoplasms are negative.	12.4	6	0.052	ND		26	0.2
77	w	68	Diabetes mellitus, creatinine 1.4 mg/dL. Left below knee amputation. Hgb 10.4 Trc 323	15.7	6	0.04	ND		40	0.1
78	m	68	621 neutrophils/μL Hgb 14.2 Trc 210	2.7	6	0.219	Y	1.5	73	0.3
79	m	57	LGL/μL Nicotine and marihuana abuse. Hgb 16.5 Trc 260	14.4	6	0.039	ND		39	0.1

80	m	68	Bicytopenia, 1,008 LGL/ μ L Hgb 14.5 Trc 134	4.2	6	0.14	ND		70	0.2
81	m	54	Leukocytosis, smoker Hgb 16.2 Trc 254	12.1	8	0.07	ND		35	0.2
82	m	42	Mycosis fungoides Hgb 14.8 Trc 252	7.3	8	0.108	ND		54	0.2
83	w	54	Urinary infection. Traumatic brain injury. 1,134 LGL/ μ L Hgb 13.0 Trc 254	12.6	8	0.064	ND		64	0.1
84	w	49	Chronic obstructive lung disease. Hgb 13.9 Trc 326	12.5	9	0.076	ND		38	0.2
85	m	76	Primary skin follicular lymphoma. Hgb 15.6 Trc 218	15.2	9	0.061	ND		61	0.1
86	m	27	Hypogammaglobulinemia, <i>Candida albicans</i> infection of the pharynx. Hgb 10.9 Trc 447	11.7	9	0.078	ND		26	0.3
87	m	56	Anemia of chronic inflammation. Hgb 12.0 MCV 93.5 fL Trc 211	13.3	11	0.084	ND		21	0.4
88	m	66	Nonalcoholic steatohepatitis. Hgb 16.9 Trc 281. 453 polyclonal B cells/ μ L, 903 eosinophils/ μ L	12.9	13	0.102	ND		34	0.3
89	m	35	Cat scratch disease Hgb 14.7 Trc 163	10.7	13	0.124	ND		31	0.4

Note: #=number.

Abbreviations: G, gender; w, woman; m, man; Hgb, hemoglobin (g/dL); Trc, thrombocytes ($10^3/\mu$ L); FISH, fluorescence in situ hybridization; i.v., intravenous; MDS, myelodysplastic syndrome; MLD, multilineage dysplasia; B-CLL-B-cell chronic lymphocytic leukemia; WBC, white blood count ($10^3/\mu$ L); BM, bone marrow; Y, yes; ND, not done; NA, not applicable (bone marrow smear or touch preparation of insufficient quality to do differential count); Lymphs, lymphocytes; Monos, monocytes; Ly/Mono, lymphocytic/monocytic.

For one patient flow cytometric study of the peripheral blood was performed on three occasions (Table 2 #46, #47, #67), and in three patients on two occasions each (#56, #57) and (#61, #72) Table 2 and in Table 1 (#5, #12).

Bone Marrow Evaluation and Clinical Information

Clinical and demographic information for each patient in this study, to include age, gender, and clinical diagnosis, was obtained via review of the electronic health record. In addition, the bone marrow pathology was reviewed, when available, as well as the corresponding cytogenetic data. Based on the sum of the clinicopathologic data, patients were classified into two groups as having either “expansive” or “nonexpansive” bone marrow process. In our study, expansive marrow process, refers to marrow involvement by an expansive abnormal pathological process such as metastases, lymphoma, leukemia, myelodysplasia, myeloproliferative processes, sarcoidosis, granulomatous, and inflammatory processes secondary to marrow involvement by infection. Myeloid hyperplasia due to pneumonia, erythroid hyperplasia due to B12 deficiency, or megakaryocytic hyperplasia in immune thrombocytopenia are not considered expansive marrow processes (atypically expansive) by our definition.

Statistical Analysis

Student's *t*-test function for independent samples was used for statistical analysis. Relationships demonstrating a *P*-value of 0.05 or less were considered statistically significant. Positive predictive value was calculated according to standard formula:

Positive predictive Value = (number of true positives)/(number of true positives + number of false positives).

Negative predictive value was calculated according to standard formula:

Negative Predictive Value = (number of true negatives)/(number of true negatives + number of false negatives).

Results

Detailed characteristics of the patients, their diagnoses, hematological parameters, and results of flow cytometric studies are presented in Table 1 and Table 2. The essential findings are as follows: the average absolute number of CD34⁺ cells in the peripheral blood in patients in whom these cells were detectable (138 flow cytometric studies) by flow cytometric method was 11 CD34⁺ cells/μL varying in a wide range (standard deviation 17.264) from less than 1 cell/μL to 147 cells/μL. The average absolute number of CD34⁺ cells in patients (49 flow cytometric studies) with an expansive (or presumed expansive) process (neoplastic or inflammatory) in the marrow was 25 cells/μL, and in patients (89 flow cytometric studies) without an expansive marrow process (or presumed negative) was 4 cells/μL (*P*<0.00007). The average percentage of CD34⁺ cells was 0.098% of leukocytes ranging from 0.01% to 0.715%. The average percentage of CD34⁺ cells in patients with bone marrow involved with expansive process or presumably involved was 0.171% and in patients with marrow not involved (or presumably not involved) was 0.057% (*P*<0.00008).

The lowest absolute number of CD34⁺ cells in our study was 0.38 cells/μL or 38 CD34⁺ cells per 100 μL of blood or 0.021% of leukocytes. The patient was a 60-year-old woman with pancytopenia and 1,800 leukocytes/μL (patient #1 Table 2). The highest absolute number was 147 CD34⁺ cells/μL or 0.705% of leukocytes. The patient was an 83-year-old man with primary myelofibrosis (JAK-2 positive) and 20,800 leukocytes/μL (#49 Table 1). The lowest percentage of CD34⁺ cells was 0.01% of peripheral blood leukocytes. The patient was a 59-year-old man with less than 1 CD34⁺ cell/μL who had had stem cell transplantation five years earlier because of precursor B cell acute lymphoblastic leukemia (#3 Table 2). The highest percentage was 0.715%, in an 88-year-old woman with non-Hodgkin lymphoma (#37 Table 1). In a 26/27-year-old woman with hidradenitis suppurativa, flow cytometric study of the peripheral blood was performed three times in a 2-year period (studies #46, #47, and #67 Table 2). The absolute numbers of CD34⁺ cells were 3 cells/μL, 3 cells/μL, and 5 cells/μL, respectively. In a 65/67-year-old woman with microcytic erythrocytes and minimal lymphocytosis, flow cytometric analysis of the peripheral blood documented four CD34⁺ cells/μL on both occasions in a two-year period (studies #56 and #57 Table 2). In a 75/76-year-old woman with refractory anemia with excess blasts-2 and monosomy-7 (studies #5 and #12, Table 1), flow cytometric analysis of the peripheral blood documented three CD34⁺ cells/μL and five CD34⁺ cells/μL in 2-month period respectively. A 46-year-old woman with bicytopenia (hemoglobin 6.9 g/dL, 2500 leukocytes/μL) had by manual count on the peripheral blood smear 1% myeloblasts (she was not included in our study), thus 25 myeloblasts/μL. Her flow cytometric study of the peripheral blood documented 18 CD34⁺ cells/μL.

A comprehensive analysis of patients' clinical findings, hematological parameters, and bone marrow findings enabled us to separate patients into two distinct groups. One group of patients (48 patients, 49 testings) had bone marrow involvement by an expansive process (proved by bone marrow biopsy) or presumed involvement (if bone marrow was not performed) on the basis of clinical picture, imaging studies, hematological parameters, and nature of the patient's disease. The other group of patients (85 patients, 89 testings) had marrow free of expansive process (excluding reactive marrow hyperplasia) or presumed free of expansive process. The average absolute number of CD34⁺ cells in the patients with an expansive process or presumed expansive process in the marrow was 25 cells/ μ L, while in the group without expansive processes it was four cells/ μ L. The difference was statistically significant ($P < 0.00007$). Cut-off 12 CD34⁺ cells/ μ L had 93% positive predictive value and 78% negative predictive value in differentiating patients with bone marrow involvement or presumably bone marrow involvement by an expansive process, versus patients with bone marrow noninvolvement by an expansive or presumably expansive process.

Discussion

Our analysis of 138 studies in 133 patients (62 women, 71 men) with less than 1% of myeloblasts in the peripheral blood by standard manual count on glass slide smears demonstrated that flow cytometric analysis can accurately obtain the absolute number and percentage of the CD34⁺ positive cells in the peripheral blood. Our methodology adds to those previously described showing that flow cytometry provides a robust, accurate, and sensitive means of enumerating CD34⁺ cells in the peripheral blood and that this value within an individual remains rather constant over time.³ The stability of this value further validates the use of flow cytometry to accurately obtain and apply these data toward a clinical endpoint.

Several methods of quantification of CD34⁺ cells in the peripheral blood have been described (Milan-Mulhouse, ISHAGE, Sison, Procount),⁸ including guidelines from the International Society of Hematotherapy and Graft Engineering (ISHAGE Guidelines)⁹ as well as modifications of this protocol.⁵ However, these protocols were primarily designed to quantify CD34⁺ cells in bone marrow transplant patients. Variations of flow cytometric methods included alterations in gating strategy, mainly CD45 versus right angle side light scatter was used, number of fluorochromes, and/or standardized beads with the intent of enumerating the number of progenitor cells within a donor product.^{10,11} Our method is conceptually similar but not identical to the Milan-Mulhouse method and the ISHAGE suggested method. However, our method employs a simpler gating strategy that allows for testing and subsequent calculations to be more easily performed. Our gate drawn on the forward scatter vs side scatter dot plot excluded cell detritus and was broad enough not to miss CD34⁺ cells that may vary in size or might not express enough of CD45 to be gated on CD45/side scatter dot plot. Nonspecific binding of CD34 to cell dusts was eliminated since only cluster or cloud of CD34 brightly positive events were regarded as real and used for quantification of CD34⁺ cells. Moreover, since no CD3⁺ lymphocytes, CD19⁺ lymphocytes or CD14⁺ monocytes co-expressed CD34 coupled with APC were counted, and since CD1a coupled with APC was 0%, it is unlikely that CD34⁺ events in the targeted gate were contaminated by CD34⁺ nonspecific and/or nonmyeloblast/stem cell events. The CD45 versus right-angle light scatter gating is the most used approach to blast isolation and quantification in bone marrow.^{12,13} However, according to the literature and our findings, forward-angle light scatter versus right angle-light scatter gating strategy is ideal for peripheral blood analysis.¹⁴

Our results are remarkably similar to those obtained by other authors using different but similar methods. There is a consensus that, in normal individuals, the percentage of CD34⁺ cells in the peripheral blood is very low, ranging from 0.01% to 0.1%, or an absolute count of 0.6 to 6 CD34⁺ cells/ μ L in a patient with a WBC count of 6,000 for example.^{5,9} The median number of CD34⁺ cells in the peripheral blood determined by an ISHAGE-guided flow cytometric study was 2.3 cells/ μ L in control subjects and 114 cells/ μ L in patients with primary myelofibrosis.¹⁵ Our findings similarly showed a low-level of circulating CD34⁺ cells in the patient group that, while not normal had nonexpansive bone marrow disorders but had hematological abnormalities that necessitated a flow cytometric study (average absolute number of four and mode of two CD34⁺ cells/ μ L).

Our study showed that determining the absolute number of CD34⁺ cells in the peripheral blood predicted whether the bone marrow would likely be involved by an expansive process or not. The difference between the average absolute numbers of CD34⁺ cells in each of group of patients was statistically significant ($P < 0.00007$), with little overlap between

the two populations. For example, in a 70-year-old patient with small cell lung cancer (patient #33 Table 1) in whom bone marrow biopsy was not performed and who had 22 CD34⁺ cells/ μ L, we can conclude that his bone marrow was involved by metastases. We can reach the same conclusion for a 63-year-old man with prostate cancer (patient #32 Table 1) who had 18 CD34⁺ cells/ μ L. By the same token we can conclude that a 70-year-old man with low normal number of platelets (patient #18 Table 2) who had had nephrectomy for renal cell carcinoma three years ago did not have bone marrow metastases since he had two CD34⁺ cells/ μ L in the peripheral blood. The absolute number of CD34⁺ cells in the peripheral blood of normal adult volunteers according to literature² varied from about one to five CD34⁺ cells/ μ L. According to our study, in a context of clinical data and laboratory parameters, the absolute number of CD34⁺ cells in the peripheral blood may therefore have a significant impact on the decision to perform bone marrow biopsy which is a painful, invasive, and costly procedure. For example, our data show that in a patient with carcinoma in whom bony metastasis is suspected, a bone marrow biopsy may be avoided, and patient presumed to have metastasis if the number of CD34⁺ cells is elevated (12 or greater CD34⁺ cells/ μ L), so long as these data support the clinical and radiologic impression. In addition to avoiding a procedure for the patient, another perceived advantage of using this method of determining bone marrow involvement is the inevitable possibility of sampling error when the lesion is focal and may be missed on a limited core biopsy. The absolute number of CD34⁺ in the peripheral blood in certain clinical settings might serve as additional information for cancer staging purposes; however, additional studies to validate our data would clearly be necessary.

To gain a better understanding of the factors that influence the numbers of peripheral blood blasts, we evaluated the relationship between the flow cytometry results, the white blood cell count and the number of bone marrow blasts. A positive correlation was identified between the number of leukocytes and absolute number of CD34⁺ cells in the peripheral blood (Pearson's coefficient correlation=0.6971). This relationship seems reasonable as the cytokines which participate in the recruitment of leukocytes resulting in leukocytosis do so, in part, by acting on bone marrow cells in the same way administered cytokines act to mobilize CD34⁺ stem cells for allograft and auto transplantations. However, in settings where no clear association to a cytokine-driven process is evident, other pathologic mechanisms may be present, such as the expansive processes described in our study. Whether physical or biochemical in nature, these pathologic processes may alter the microenvironment of the bone marrow, allowing the inappropriate release of immature cells into the peripheral blood. The 74-year-old man (#41 Table 1) with severe lung infection with *Cryptococcus* also had *Cryptococcus* meningitis with numerous Cryptococci in histiocytes in the cerebrospinal fluid. In his peripheral blood were 23,800 WBC/ μ L and 38 CD34⁺ cells/ μ L. Since the percentage of his CD34⁺ cells was 0.16% of leukocytes, similar to the average percentage in patients with marrow involvement by an expansive process (0.171%), we inferred that the increased number of CD34⁺ cells (38 cells/ μ L) was due to bone marrow involvement by infection (inflammatory tumor) with *Cryptococcus* (bone marrow was not done) and not only to leukocytosis per se. Thus, percentage of CD34⁺ cells in the peripheral blood is also a useful parameter. The average percentage of CD34⁺ cells in the peripheral blood of our 138 studies was 0.098% ranging from 0.01% to 0.715%. The average percentage of patients with expansive marrow process or presumably having expansive process was 0.171% and of patients with marrow not involved or presumably not involved by an expansive process was 0.057% ($P<0.00008$). Interestingly, the absolute number of CD34⁺ cells in the peripheral blood did not correlate with the percentage of myeloblasts in the bone marrow (coefficient correlation was -0.23219). These data suggest the number of circulating blasts is less reflective of the bone marrow differential and more dependent upon the presence of pathology that may disrupt the regulatory processes which govern the release of cells into the peripheral blood.

We have chosen cutoff value of 12 CD34⁺ cells/ μ L in the peripheral blood to differentiate patients with marrow involvement with expansive versus nonexpansive processes because its positive predictive value was 93% and negative predictive value 78%. However, several outliers were discovered in our study. In a small number of patients, the absolute number of CD34⁺ cells in the peripheral blood overlapped between the groups in our study. For example, several outliers with low absolute number of CD34⁺ cells in the peripheral blood had marrow involved by an expansive process. However, fewer numbers of patients showed the converse (only two out of 89 patients) without marrow involvement (or presumably without involvement) had slightly increased numbers of CD34⁺ cells. Patient #1 Table 1 (a 28-year-old woman with anaplastic large cell lymphoma) and one CD34⁺ cell/ μ L had only four malignant cells in the bone marrow,

not obvious in the hematoxylin and eosin-stained core biopsy but demonstrated by an immunoperoxidase stain for CD30. In her case we can suggest that the minuscule amount of expansive process was related to low absolute number of CD34⁺ cells in the peripheral blood. We have no explanation why patient #6 Table 1 (79-year-old man) who had a 95% marrow replacement by B cell chronic lymphocytic leukemia cells had only three CD34⁺ cells/ μ L while patient #45 Table 1 (72-year-old man) with the same disease had 80 CD34⁺ cells/ μ L. Patient #8 Table 1 (65-year-old man with pancytopenia and myelodysplastic syndrome with multilineage dysplasia) had three CD34⁺ cells/ μ L while patient #31 Table 1 (48-year-old woman) with the same disease had 17 CD34⁺ cells/ μ L.

Here are four possible mechanisms for (outliers) discrepancy between the expansive or nonexpansive marrow status and absolute number of CD34⁺ cells in the peripheral blood. (1) Minimal bone marrow involvement by tumor prevents significant disruption in the normal release of CD34⁺ cells and leads to falsely low result. (2) Treatment-related clearance of CD34⁺ cells might also lead to false low result. (3) Unrecognized pathology in the bone marrow (for example biopsy missed neoplastic lesion) may result in unexpectedly high absolute number of CD34⁺ cells in the peripheral blood but be actually correct. (4) Treatment-related damage to the bone marrow environment might lead to inappropriate release of CD34⁺ cells and false positive results.

Patient #36 Table 1, a 66-year-old man with mantle cell lymphoma involving 70% of the marrow core biopsy, had 24 CD34⁺ cells in the peripheral blood and after treatment there were no detectable CD34⁺ cells in the peripheral blood. We could conclude that the clearance of CD34⁺ cells from the peripheral blood after treatment, as detected by flow cytometry may be indicative of response to the treatment and that marrow became free of involvement by mantle cell lymphoma. Bone marrow biopsy was not performed. Since there were no CD34⁺ cells in the peripheral blood, this patient's second study was not included in this research or our statistics. Given these findings, the number of CD34⁺ cells should not be regarded as absolutely decisive, but rather as another data point that should be integrated in the patient's whole clinical, laboratory, and radiological picture. With knowledge of these limitations, we recommend that determination of the absolute number and percentage of CD34⁺ cells obtained by flow cytometric analysis of the peripheral blood be performed with intent of using these values to predict bone marrow pathology and ultimately help in the determination of the necessity for bone marrow biopsy.

We have found that multiparameter flow cytometric testing by simple gating mononuclear cells can accurately quantify the absolute number of CD34⁺ cells in the peripheral blood. The method is extremely sensitive and can detect fewer than one CD34⁺ cells/ μ L. Quantification of the CD34⁺ cells in the peripheral blood by flow cytometric analysis is a useful parameter that may predict whether the bone marrow is involved or not by an expansive process, when evaluated in concert with the clinical picture, laboratory data, and imaging findings. Based on the results of our study, flow cytometry of peripheral blood is recommended as these data may provide a useful threshold in the decision-making process to perform a bone marrow biopsy. As part of the initial evaluation, flow cytometric enumeration of peripheral CD34⁺ cells may play an important role in directing the hematologic evaluation of patients when circulating blasts are not seen on peripheral blood smear. The absolute number of 12 or more CD34⁺ cells/ μ L in the peripheral blood favors marrow involvement by an expansive process despite a negative bone marrow biopsy, suggesting possibility of inadequate sampling procedure which may have missed the lesion.

Conclusions

1. Flow cytometric method is very sensitive, can accurately detect in the peripheral blood less than one CD34⁺ cells/ μ L, and in our study it demonstrated that the average absolute number of CD34⁺ cells in the peripheral blood in patients with less than 1% blasts by manual count was 11 CD34⁺ cells/ μ L.
2. The average absolute number of CD34⁺ cells in the peripheral blood in patients with expansive pathologic process (or presumed expansive) is 25 CD34⁺ cells/ μ L. It is significantly higher ($P < 0.00007$) than in patients without marrow pathologic expansive processes, four CD34⁺ cells/ μ L, and thus, absolute number of CD34⁺ cells can help make diagnosis regarding marrow involvement.
3. The absolute number CD34⁺ cells in the peripheral blood can help to decide whether or not to do bone marrow biopsy.

4. The absolute number of 12 or more CD34⁺ cells/ μ L in the peripheral blood might suggest bone marrow involvement despite a negative bone marrow biopsy for cancer staging purpose which, may have missed focal lesion.
5. We suggest that other clinicians use our simple method of counting absolute number of CD34⁺ cells in the peripheral blood by flow cytometry and confirm our findings. The aims, data and analysis of our study support the conclusion, essentially that this method offers insight into the likelihood of a significant underlying bone marrow process, and with this additional (rapidly available) information, clinicians may be able to determine the appropriateness of bone marrow biopsy in the management of their patients.

Ethical Approval

Research has been carried out within an appropriate ethical framework in accordance with COPE guidelines approved by (Charleston Area Medical Center, Charleston West Virginia/West Virginia University) CAMC/WVU Charleston Division Institutional Review Board (IRB), approval number 20-680 on June 23, 2020. Regarding human participants a waiver of consent was requested at the time of study application and CAMC/WVU Charleston Division IRB granted (approval number 20-680) an exemption from requiring written informed consent, a waiver of consent 45 CFR 46.116 (f), as it was determined the use of retrospective data posed “not greater than minimal risk” to participants, and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Arber DA, Orazi A, Hassserijan RP, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200–1228. doi:10.1182/blood.2022015850
2. Sutherland DR, Keating A, Nayar R, Anania S, Stewart AK. Sensitive detection and enumeration of CD34 positive cells in peripheral blood and cord blood by flow cytometry. *Exp Hematol*. 1994;22(10):1003–1010.
3. Eidenschink L, DiZerega G, Rodgers K, Bartlett M, Wells DA, Loken MR. Basal levels of CD34 positive cells in peripheral blood differ between individuals and are stable for 18 months. *Cytometry B Clin Cytom*. 2012;82(1):18–25. doi:10.1002/cyto.b.20611
4. Bender JG, Unverzagt KL, Walker DE, et al. Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. *Blood*. 1991;77(12):2591–2596. doi:10.1182/blood.V77.12.2591.2591
5. Kikuchi-Taura A, Soma T, Matsuyama T, Stern DM, Taguci A. A new protocol for quantifying CD34⁺ cells in peripheral blood of patients with cardiovascular disease. *Tex Heart Inst J*. 2006;33(4):427–429.
6. Siena S, Bregni M, Brando B, et al. Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. *Blood*. 1991;77(2):400–409. doi:10.1182/blood.V77.2.400.400
7. Kutok JL, Roma AO, Lemire SJ, Dorfman DM. Four-color flow cytometric immunophenotypic determination of peripheral blood CD4⁺ T-lymphocyte counts: a comparison of validity and cost-effectiveness with a two-color method. *Am J Clin Pathol*. 1998;110(4):465–470. doi:10.1093/ajcp/110.4.465
8. Gratama JW, Orfao A, Barmett D, et al. Flow cytometric enumeration of CD34⁺ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis. *Cytometry*. 1998;34(3):128–142. doi:10.1002/(SICI)1097-0320(19980615)34:3<128::AID-CYTO3>3.0.CO;2-D
9. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Jee I. The ISHAGE guidelines for CD34⁺ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother*. 1996;5(3):213–226. doi:10.1089/scd.1.1996.5.213
10. Gajkowska A, Oldak T, Jastrzevska M, et al. Flow cytometric enumeration of CD34⁺ positive hematopoietic and progenitor cells in leukapheresis product and bone marrow for clinical transplantation: a comparison of three methods. *Folia Histochem Cytobiol*. 2006;44(1):53–60.
11. Sutherland DR, Nayyar R, Acton E, Giftakis A, Dean S, Mosiman VL. Comparison of two single-platform ISHAGE-based CD34 enumeration protocols on BD FACSCalibur and FACSCanto flow cytometers. *Cytotherapy*. 2009;11(5):595–605. doi:10.1080/14653240902923161
12. Kroft SH, Karandikar NJ. Flow cytometric analysis of acute leukemias, myelodysplastic syndromes, and myeloproliferative disorders. In: Carey JL, McCoy JP, Keren DF, editors. *Flow Cytometry in Clinical Diagnosis*. 4th ed. Chicago, IL: American Society for Clinical Pathology; 2007:168–214.
13. Harrington AM, Olteanu H, Kroft SH. A dissection of the CD45/side scatter “blast gate”. *Am J Clin Pathol*. 2012;137(5):800–804. doi:10.1309/AJCPN4G1IZPABRLH
14. Sun T, Sangaline R, Ryder J, et al. Gating strategy for immunophenotyping of leukemia and lymphoma. *Am J Clin Pathol*. 1997;108(2):152–157. doi:10.1093/ajcp/108.2.152
15. Passamonti F, Vanelli L, Malabarba L, et al. Clinical utility of the absolute number of circulating CD34-positive cells in patients with chronic myeloproliferative disorders. *Haematologica*. 2003;88(10):1123–1129.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Correlation Between Hypercoagulable State and Severity Level of Ischemic Stroke With Covid-19 Infection

Lisda Amalia

To cite this article: Lisda Amalia (2023) Correlation Between Hypercoagulable State and Severity Level of Ischemic Stroke With Covid-19 Infection, Journal of Blood Medicine, , 537-542, DOI: [10.2147/JBM.S429357](https://doi.org/10.2147/JBM.S429357)

To link to this article: <https://doi.org/10.2147/JBM.S429357>



© 2023 Amalia.



Published online: 11 Oct 2023.



Submit your article to this journal



Article views: 190



View related articles



View Crossmark data

Correlation Between Hypercoagulable State and Severity Level of Ischemic Stroke With Covid-19 Infection

Lisda Amalia 

Department of Neurology, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia

Correspondence: Lisda Amalia, Department of Neurology, Medical Faculty, Universitas Padjadjaran, Jl. Eykman 38, Bandung, 40161, Indonesia, Email dr.lisda@gmail.com

Background: Hypercoagulable state in acute ischemic stroke patients with COVID-19, was found to occur in most cases, may affect the severity and clinical outcome of acute ischemic stroke with COVID-19. Ischemic stroke patients with COVID-19 infection have worsen prognosis in mortality regarding hypercoagulable state condition.

Objective: The study aims to determine the relationship between the hypercoagulable state and the severity of acute ischemic stroke patients with COVID-19.

Methods: This study is a retrospective analytic study using a cross-sectional method in acute ischemic stroke who meet the criteria must have focal clinical symptoms or global dysfunction lasting more than 24 hours, be caused by vascular factors, be confirmed positive for COVID-19, NIHSS (admission and discharge), and have an examination of D-dimer and/or fibrinogen. Chi-Square is used for data processing relationship analysis.

Results: A total of 32 patients met the inclusion and exclusion criteria of this study. Elevated D-dimer and/or fibrinogen were found in 28 patients (87.5%), confirming a hypercoagulable state. In this study, the average value of D-dimer was 5.3 mg/mL, and fibrinogen was 479 mg/dL. Based on the admission NIHSS score, it was found that most of the patients had moderate strokes with an average NIHSS score of 12. The chi-square test results showed no relationship between the hypercoagulable state and the severity of acute ischemic stroke as measured by NIHSS admission ($p=0.333$), but it was closely related to NIHSS exit ($p=0.02$). The finding supports that 40.62% of acute ischemic stroke patients with COVID-19 confirmed to have a hypercoagulable state had a death discharge status.

Conclusion: There is no significant relationship between hypercoagulable state and stroke severity on admission, but it closely related to NIHSS on discharge and high mortality in acute ischemic stroke patients with COVID-19.

Keywords: hypercoagulable state, acute ischemic stroke, degree of severity, NIHSS

Introduction

Stroke is defined by the World Health Organization (WHO) as a sudden decline in focal or global neurological function that develops rapidly and lasts more than 24 hours or causes death caused by vascular disorders. Stroke is the biggest cause of death and disability in the world. Based on data from the World Stroke Organization (WSO) for 2022, more than 12 million new stroke events occur yearly, with a death rate of 6.5 million.¹ After entering the COVID-19 pandemic, it was found that stroke accounts for 1–6% of all complications occurs due to infection with COVID-19. Acute ischemic stroke, which is defined as a decrease in blood supply to the brain and causes neurological deficits, is the most common cerebrovascular complication (CVD) in COVID-19 patients.² Increased coagulation factors were found in acute ischemic stroke patients with COVID-19, indicating a relationship between hypercoagulable states due to COVID infection -19 with acute ischemic stroke and also influence for improving clinical outcome.³

Differences in characteristics of stroke patients with and without Covid-19, among others, stroke patients with COVID-19 showed more large vessel occlusion (LVO) (17.9% Covid-19, 8.1% non-Covid-19), experienced a degree more severe stroke

according to NIHSS (score 8 Covid-19, score 5 non-Covid-19), higher D-dimer value, higher degree of disability (Rankin score) (score 4 Covid-19, score 3 non-Covid-19), and higher mortality (19.8% Covid-19, 6.9% non-Covid-19).⁴

The National Institute of Health Stroke Scale (NIHSS) is a tool to qualitatively and systematically measure the severity of stroke by measuring neurological functions, including levels of consciousness, sensory response, motor movements, speech and communication skills, and attention. A high NIHSS score indicates worse neurological decline. In acute ischemic stroke patients with COVID-19, the NIHSS score was found to be higher compared to acute ischemic stroke patients without COVID-19.^{3,5} This study aims to determine the relationship between hypercoagulable conditions and the degree of severity in acute ischemic stroke patients with COVID-19.

Methods

The subjects of this study were obtained from the medical records of patients treated at the Department of Neurology at Dr. Central General Hospital. Hasan Sadikin Bandung in the period March 2020 – December 2022. These patients must have focal clinical symptoms or global dysfunction lasting more than 24 hours and caused by vascular factors. COVID-19 infection status was confirmed positive using a Real-Time Polymerase Chain Reaction (RT-PCR) test. The hypercoagulable state is determined by increased D-dimer values (normal values ≤ 0.55 mg/mL) or increased fibrinogen levels (normal values 238–498 mg/dL). Impaired neurological function was assessed using the National Institute of Health Stroke Scale (NIHSS). NIHSS scores are classified into four categories: mild stroke (1–4), moderate stroke (5–15), moderate stroke (16–20), and severe stroke (21–42). The Trial of Organization 10,172 in Acute Stroke Treatment (TOAST) classification is used to determine the causes and mechanisms of stroke, namely large-artery atherosclerosis (LAA), cardioembolism (CE), small-vessel occlusion (SVO), stroke of other determined etiology, and stroke of undetermined etiology. Patient demographic information, risk factors, and clinical outcomes were recorded. Subjects excluded were patients with hemorrhagic stroke, patients without NIHSS scores on admission, and patients with poor medical records. Sampling using the total sampling method. Chi-Square is used for data processing relationship analysis.

Subjects who met the inclusion criteria expressed their willingness to participate in this study by signing informed consent after obtaining an explanation. The ethics committee of Universitas Padjadjaran Bandung received the research ethics approval under the ethical clearance number 612/UN6.KEP/EC/2023 and complies with the Declaration of Helsinki. Writing informed consent was obtained from the study participants.

Result

Of the 545 acute ischemic stroke patients treated at Dr. Hasan Sadikin Bandung during the vulnerable period of March 2020 – December 2022, 32 patients were declared to have met the research criteria. Most patients were women, over 55, and had moderate strokes (NIHSS mean=12). Hypertension is the biggest risk factor (78.1%), followed by dyslipidemia (46.9%) and heart disease (40.6%). Table 1 shows the characteristic distribution of acute ischemic stroke patients with COVID-19.

Table 1 Characteristics of Research Subjects

Variable	(n)	(%)
Age (n = 32)		
35–44	2	6.25
45–54	5	15.62
55–64	12	37.5
65–74	10	31.25
> 75	3	9.37
Gender (n = 32)		
Male	13	40.6
Female	19	59.4
Risk factor (n = 32)		
Hypertension	25	78.1
Dyslipidemia	15	46.9

(Continued)

Table 1 (Continued).

Variable	(n)	(%)
Cardiac problem	13	40.6
Diabetes Mellitus	12	37.5
Smoking	10	31.3
History of stroke	10	31.3
Hyperuricemia	1	3.1
NIHSS on admission (n = 32)		
Mild Stroke	2	6.25
Moderate Stroke	19	59.37
Moderate to severe Stroke	10	31.25
Severe Stroke	1	3.12
D-dimer (n = 31)		
≤ 0.55 mg/mL	5	16.12
> 0.55 mg/mL	26	83.87
Fibrinogen (n = 26)		
≤ 498 mg/dL	16	48.38
> 498 mg/dL	10	32.25
TOAST Classification (n = 32)		
Large artery atherosclerosis	2	6.25
Cardioembolism	2	6.25
Stroke of other determined etiology	26	81.25
Stroke of undetermined etiology	2	6.25

The hypercoagulable state was confirmed in 28 patients (87.5%). Increased D-dimer was found in 83.87%, while increased fibrinogen was found in 38.46%. The average D-dimer and fibrinogen values were 5.3 mg/mL and 479 mg/dL. 81.25% of patients had strokes of other determined etiology based on the TOAST classification. The average NIHSS admission score with hypercoagulable conditions was 13.32 (moderate stroke), while patients without hypercoagulable conditions were 7.5 (moderate stroke). There were 40.62% of patients were discharged dead. Most had higher NIHSS admission scores and were confirmed to be hypercoagulable. Table 2 shows the relationship between the hypercoagulable state and the severity of acute ischemic stroke with COVID-19.

Based on the chi-square test, we found no relationship between the hypercoagulable state and the severity of acute ischemic stroke in COVID-19 patients at admission ($p=0.333$). It was found that the hypercoagulable state was associated with the severity of acute ischemic stroke using NIHSS on discharge. ($p=0.02$).

Table 2 Correlation Between Hypercoagulable State with NIHSS

NIHSS Score	Hypercoagulable State		P-value
	Yes	No	
NIHSS on admission (n = 32)			0.333
Mild Stroke	1	1	
Moderate Stroke	17	3	
Moderate to severe Stroke	9	—	
Severe Stroke	1	—	0.022
NIHSS on discharge (n = 26)			
Mild Stroke	4	—	
Moderate Stroke	3	3	
Moderate to severe Stroke	1	—	
Severe Stroke	—	—	
Death	13	—	

Discussion

Coagulopathy is one of the clinical manifestations of severe Coronavirus Disease of 2019 (Covid-19), and causes a poor prognosis of the disease. Covid-19 patients experience a severe inflammatory state that causes an increase in the activation of the coagulation cascade, an increase in thrombin, and a hypercoagulable state, thereby increasing the risk of thrombosis in the blood vessels of the brain and spinal cord. Acute ischemic stroke accounts for 1–6% of all complications due to COVID-19.^{2,6,7} Although there are several reports of strokes in young COVID-19 patients, we found that the average age of acute ischemic stroke patients with COVID-19 is 61.6, and the most common risk factor was hypertension (78.1%). This is consistent with research by Hidayat et al and Luo et al, who reported 69% and 66% yields, respectively.^{8,9}

Based on the TOAST classification, most of our subjects had strokes of other determined etiology (81.25%). Luo et al reported consistent results, namely cryptogenic stroke in 35% of acute ischemic stroke patients with COVID-19. This is consistent with a study by Yaghi et al, who found that stroke patients with COVID-19 were more likely to experience cryptogenic strokes, suggesting a specific mechanism for developing acute ischemic stroke in patients with COVID-19.^{3,8}

The most frequently suggested mechanism of acute ischemic stroke in COVID-19 patients is a hypercoagulable state due to the coagulation cascade caused by the immune response to COVID-19 infection.³ In line with other studies, hypercoagulable state was also confirmed in most of our patients (87.5%). An increase in D-dimer which was considered a consistent laboratory finding in COVID-19 patients was confirmed in 83.87% while an increase in fibrinogen was found in 38.46%.¹⁰ This is supported by research by Hidayat et al who reported an increase in D-dimer in 98.1% of stroke patients at the University of Indonesia Hospital.¹¹

In our study, the mean and median D-dimer were 5.36 mg/mL and 2.36 mg/mL, respectively, well above the normal value of 0.55 mg/mL. Yaghi et al in a New York study found similar results, confirming D-dimer with a median of 3.91 mg/mL in acute ischemic stroke patients with COVID-19.³ In Spain, Fàbregas found slightly lower results (median: 1.76 mg/mL).⁹ Based on several previous studies, increased D-dimer is associated with higher mortality.¹²

Based on a systematic review by Tan et al, COVID-19 patients were found to have moderate to severe strokes.¹³ In this study, the National Institute of Health Stroke Scale was used at admission and most of the subjects had moderate strokes (12.5) which is consistent with findings. Hivaneon's et al reported a mean NIHSS score of 9.7 and Nannoni et al in their systematic review (median: 5).^{6,14} Compared with stroke patients without COVID-19, acute ischemic stroke patients with COVID-19 also showed higher NIHSS scores on admission.^{3,6} This is why we hypothesize that hypercoagulable conditions will affect the severity of acute ischemic stroke in patients with COVID-19.

Based on our study, patients with hypercoagulable states showed a mean NIHSS score on admission of 13.32, while patients without hypercoagulable conditions showed a mean score of 7.5. These findings show a linear relationship between hypercoagulable state and stroke severity on admission. However, we found no significant relationship between the hypercoagulable state and the severity of acute ischemic stroke at admission ($p=0.333$). We suspect that higher NIHSS scores on admission in acute ischemic stroke patients with COVID-19 in some studies are due to the severity of COVID-19 infection and other patient risk factors. As most patients with higher admission NIHSS scores were found to have cardioembolic strokes, we consider that acute ischemic stroke is exacerbated by the hypercoagulable state resulting from COVID-19 infection, not solely its cause.²

It was previously known that D-dimer above 1 mg/mL on admission was associated with a higher probability of in-hospital death and was a predictor of mortality. In a study by Tang et al, non-survivors showed a D-dimer rate of 2.12 mg/mL, while survivors showed 0.61 mg/mL.¹² This is consistent with our study finding 8.29 mg/mL in non-survivors and 3.25 mg/mL in survivors. This also shows that acute ischemic stroke patients with hypercoagulable conditions have a higher risk of dying.^{2,9} The presence of Covid-19 complications such as thrombosis can affect the patient's prognosis and mortality.⁷

SARS-CoV-2 infection in several conditions can trigger an exaggerated immune reaction, otherwise known as a cytokine storm, which can activate secondary hemostasis or coagulation cascade. This causes excessive fibrin degradation product (FDG) production, reflected in the increase in D-dimer or fibrinogen in COVID-19 patients, causing

a hypercoagulable state. The coagulation cascade will form a thrombus that can travel through the vascular system, increasing the risk of acute ischemic stroke in COVID-19 patients.^{2,7}

The lack of research subjects is the main limitation of this study. D-dimer and fibrinogen tests are not done in many acute ischemic stroke patients, with COVID-19 being the main cause. This study did not consider hypercoagulable conditions due to genetic disorders or causes other than COVID-19. In addition, this study was only conducted at a central general hospital, so it cannot represent other types of hospitals. More in-depth and multicentre studies are needed to determine whether there is a relationship between hypercoagulable states and acute ischemic stroke severity in COVID-19 patients and to determine the relationship between D-dimer and acute ischemic stroke severity on admission.¹⁴

Conclusion

There was no significant relationship between the hypercoagulable state and the severity of acute ischemic stroke in COVID-19 patients at admission, but it closely related to NIHSS on discharge and high mortality in acute ischemic stroke patients with COVID-19. The presence of Covid-19 complications such as thrombosis in acute stroke can affect the patient's prognosis and mortality.

Acknowledgment

The authors would like to thank the Neurology Department of Dr. Hasan Sadikin Bandung General Hospital staff for their contributions to this study.

Disclosure

The author reports no conflicts of interest in this work.

References

1. Feigin VL, Brainin M, Norrving B, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. *Int J Stroke*. 2022;17(1):18–29. doi:10.1177/17474930211065917
2. Amalia L. Characteristic of acute ischemic stroke patients based on TOAST classification during COVID-19 pandemic era: a single centre study. *Int J Gen Med*. 2023;16:581–588. doi:10.2147/IJGM.S394017
3. Yaghi S, Ishida K, Torres J, et al. SARS-CoV-2 and stroke in a New York Healthcare System. *Stroke*. 2020;51(7):2002–2011. doi:10.1161/STROKEAHA.120.030335
4. Li Y, Li M, Wang M, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vasc Neurol*. 2020;5(3):279–284. doi:10.1136/svn-2020-000431
5. Finsterer J, Scorza FA, Scorza CA, Fiorini AC. Ischemic stroke in 455 COVID-19 patients. *Clinics*. 2022;77:100012. doi:10.1016/j.clinsp.2022.100012
6. Nannoni S, de Groot R, Bell S, Markus HS. Stroke in COVID-19: a systematic review and meta-analysis. In: *International Journal of Stroke*. Vol. 16. SAGE Publications Inc; 2021:137–149.
7. Amalia L. Kelainan Neurologi akibat Kondisi Hiperkoagulasi pada Pasien Coronavirus Disease-19. *JNI*. 2023;12(1):58–66. doi:10.24244/jni.v12i1.452
8. Luo W, Liu X, Bao K, Huang C. Ischemic stroke associated with COVID-19: a systematic review and meta-analysis. *J Neurol*. 2022;269(4):1731–1740. doi:10.1007/s00415-021-10837-7
9. Martí-Fàbregas J, Guisado-Alonso D, Delgado-Mederos R, et al. Impact of COVID-19 infection on the outcome of patients with ischemic stroke. *Stroke*. 2021;52(12):3908–3917. doi:10.1161/STROKEAHA.121.034883
10. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost*. 2020;18(5):1023–1026. doi:10.1111/jth.14810
11. Hidayat R, Widjaya N, Djulianisaa Z, et al. Ischemic stroke in COVID-19 patients: a cross-sectional study from an Indonesian COVID-19 referral hospital. *Egypt J Neurol Psychiatr Neurosurg*. 2022;58(1):93. doi:10.1186/s41983-022-00528-z
12. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*. 2020;18(4):844–847. doi:10.1111/jth.14768
13. Tan YK, Goh C, Leow AST, et al. COVID-19 and ischemic stroke: a systematic review and meta-summary of the literature. *J Thromb Thrombolysis*. 2020;50(3):587–595. doi:10.1007/s11239-020-02228-y
14. de Havenon A, Zhou LW, Yaghi S, Frontera JA, Sheth KN. Effect of COVID-19 on acute ischemic stroke severity and mortality in 2020: results from the 2020 National Inpatient Sample. *Stroke*. 2023;54(5). doi:10.1161/STROKEAHA.122.041929

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, Borana, Ethiopia

Girma Ashenafi, Melatwork Tibebu, Dagnamyelew Tilahun & Aster Tsegaye

To cite this article: Girma Ashenafi, Melatwork Tibebu, Dagnamyelew Tilahun & Aster Tsegaye (2023) Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, Borana, Ethiopia, Journal of Blood Medicine, , 543-554, DOI: [10.2147/JBM.S419414](https://doi.org/10.2147/JBM.S419414)

To link to this article: <https://doi.org/10.2147/JBM.S419414>



© 2023 Ashenafi et al.



Published online: 20 Oct 2023.



Submit your article to this journal



Article views: 176



View related articles



View Crossmark data

Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, Borana, Ethiopia

Girma Ashenafi¹, Melatwork Tibebe², Dagnamyelew Tilahun¹, Aster Tsegaye²

¹Department of Medical Laboratory Sciences, Bule Hora University, Bule Hora, Oromia, Ethiopia; ²Department of Medical Laboratory Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Correspondence: Melatwork Tibebe, Email melatwork.tibebe@aau.edu.et

Background: Immunohematological abnormalities among human immunodeficiency virus-infected patients are common abnormalities associated with severe depletion of the immune system, covering a stage of acute syndrome to an advanced disease. The greatest impact was observed in the low- and middle-income countries. However, in Ethiopia, little attention has been paid, and only limited published information exists regarding immunohematological abnormalities among individuals receiving highly active antiretroviral treatment.

Objective: This study aimed to assess changes in immunological and hematological parameters in HIV-infected patients receiving HAART for at least six months at the antiretroviral therapy clinic of Yabelo Hospital, Borena, Ethiopia.

Methods: A cross-sectional study was conducted from February to July 2021 using convenient sampling to recruit 333 participants. Sociodemographic data and clinical characteristics were collected using a pretested questionnaire. Baseline data were extracted from medical records and after six month immunohematological measurements were performed on blood samples collected during the study period. Data analysis was performed using SPSS version 25. Descriptive analysis was performed, and the results are presented as numbers and percentages or means $\pm$ SD. A paired *t*-test was used to compare the mean values of the immunohematological parameters before and after six of taking HAART. Statistical significance was set at $P < 0.05$.

Results: The prevalence of anemia, leucopenia, neutropenia, lymphopenia and thrombocytopenia were 47.4%, 73.3%, 58.3%, 76.9% and 3.3% before initiation of HAART and 23.1%, 36.4%, 23.4%, 35.7% and 2.4% after initiation of HAART, respectively; Compared to baseline, there was also a significant decrease in the rate of Immunosuppression ($CD4 < 350$) from 62.2% at base line to 20.7% after HAART initiation.

Conclusion: Immunohematological profile of the patients improved after the initiation of HAART. The observation of large proportion of immunosuppressed individuals at baseline warrants advocating for HIV testing in the pastoralist community so that infected patients could benefit from early initiation of HAART.

Keywords: HIV, highly active antiretroviral therapy, immunohematological abnormalities

Introduction

Human immunodeficiency virus (HIV) infection is characterized by severe depletion of the immune system, covering the stages of acute syndrome to an advanced disease (Acquired Immunodeficiency Syndrome-AIDS). The greatest impact is observed in low- and middle-income countries where an estimated 90% of the global HIV-infected population live; sub-Saharan Africa is the most affected area of world while South-East Asia is the second most affected.¹⁻³

Immunohematological abnormalities in HIV patients are due to diverse reasons which include immune-mediated destruction of cells, direct cytopathic effects of the virus, secondary to various infections, neoplasms, and drug toxicity.⁴ The most prevalent haematological disorder observed in adults with HIV infection is anemia. Incidences of anemia are

particularly high in patients with late stages of the disease and a reduced CD4+ T cell count. The virus replicates in T cells and in turn reduces the growth of bone marrow progenitors, thus suppressing hemopoiesis.⁵ Anemia and neutropenia are generally caused by inadequate blood cell production because of bone marrow suppression by HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment.^{6,7}

Among hematological abnormalities, neutropenia is commonly observed after development of AIDS, and has been associated with types of HAART medications used to treat HIV infection.⁸ Colony growth hormones of the progenitor cells decreased in patients taking HAART, this leads to decreased production of granulocyte and monocytes produced by the infected cells known to suppress neutrophil production.⁹ On the other hand, thrombocytopenia could be seen from increased platelets destruction and decreased platelet production by the HIV-infected megakaryocytic cells. Like other immunohematological abnormalities, decreased CD4 count also manifest due to HIV using CD4 receptor as site of attachment and replication.^{8–10}

Understanding immunohematological outcomes is essential for evaluating treatment and prognosis during the follow-up of patients with HIV. Highly active antiretroviral therapy (HAART) is a combined therapy used in HIV-positive patients to radically lengthen the time to AIDS development and/or progression to death in HIV-infected people. The major mechanism of action of HAART is the inhibition of plasma HIV levels, which promotes an increase in CD4 T-lymphocyte count and function. The aim is inhibiting viral replication while minimizing the toxicities and side effects associated with the available drugs. Using HAART, proper growth in children can be promoted, and the survival of all HIV-infected patients can be prolonged by reducing their level of illness and improving their quality of life.¹¹

HAART significantly improves immunohematological changes in HIV-infected individuals. Published data on this outcome are scarce in the Southern Region of Ethiopia, particularly in the Borana Zone. Distinguishing the changes in the immunohematological profiles during treatment can help caregivers and physicians prevent most adverse effects and adequately manage patients. Moreover, our findings can serve as reference and input material for policymakers in the study area, not to mention further research conducted in the region as well as around the globe.

Materials and Methods

Study Setting, Design and Study Population

This cross-sectional study was conducted at the Yabelo General Hospital, Borana, Ethiopia, from February to July 2021. Borena is located at 567 km South of Addis Ababa and its capital is Yabelo town. There are one governmental general hospital and one health center in the town. The hospital provides ART service for HIV positive individuals. Primary and secondary clinical data of the study participants were collected using a pretested standardized questionnaire. The sample size was calculated using a single-population proportion formula and a correction formula for a finite population, to recruit 333 study participants using a convenient sampling technique. HIV-positive patients who were receiving HAART for at least six months and provided informed consent were included in the study. However, patients taking vitamins and iron supplements at the time of sampling, pregnant women, and those who had received a blood transfusion within the last 4 months were excluded.

Data Collection and Quality Assurance

Baseline complete blood count, CD4 and other clinical and demographic data of the study participants before HAART initiation were extracted from the medical record books. Socio-demographic and clinical data pertinent to our study after at least 6 months of initiation of HAART were collected using a trained data collector. Blood samples were collected once in the mornings to minimize diurnal variation when patients come for their routine care using Ethylene diamine tetra acetic acid (EDTA) tube and complete blood count (CBC) was analyzed using Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan). BD FACS PRESTO machine (BD Biosciences, San Jose, California) was used to count CD4+ T cells to detect the immunological abnormalities. The performance of both analyzers was verified by running quality control samples. The quality of the data was assured by designing a pretested standardized questionnaire that was prepared in English and translated to Afan Oromo (native language for the people in Borana, Ethiopia). Besides, standard operating procedures (SOPs) were strictly followed and all reagents were checked for expiration dates.

Statistical Analysis

For analysis, the data were coded, cleaned, entered into EpiData version 3.1 and analyzed using SPSS version 25 (IBM® SPSS®, IBM Corp., Armonk, NY, USA). Descriptive analysis was performed, and the results are presented as numbers and percentages or means $\pm$ SD. A paired *t*-test was used to compare the mean values of the immunohematological parameters before and after six months of taking HAART. Bivariate and multivariable analyses were used to determine the associations between parameters and factors. Statistical significance was set at $p < 0.05$.

Ethical Consideration

This study was approved by the Department Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences, Addis Ababa University. After a letter of cooperation was sent to Borana Health Bureau, the Institutional Review Board of the Borana Health Bureau also approved the study and wrote support letter to Yabelo General Hospital for the study to proceed. Written informed consent was obtained from all the participants. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Socio-Demographic Characteristics

A total of 333 individuals aged 18 years and above, of whom 170 (51.1%) male and 163 (48.9%) females were included in the study, with 100% response rate. The majority of these participants were between the ages of 29–38 followed by 18–28 years with a mean age and standard deviation of 32.83 and 9.48 years. In regards to the marital status, educational level, and occupation of the participants; 151 (45.3%) of them were married, 112 (33.6%) had no formal education, and 85 (25.5%) were housewives. More than half of them were urban residents 195 (58.6%) (Table 1).

Table 1 Socio-Demographic Characteristics of HIV Patients Taking HAART for at Least 6 Months at Yabelo General Hospital, Borana Ethiopia, 2021

Variables	Frequency and Percentile Number (%)
Age	
18–28	122 (36.6)
29–38	129 (38.7)
39–48	53 (15.9)
49–58	24 (7.2)
≥ 59	5 (1.5)
Sex	
Male	170 (51.1)
Female	163 (48.9)
Religion	
Protestant	97 (36.6)
Orthodox	81 (38.7)
Muslim	79 (15.9)
Catholic	42 (7.2)
Wakefata	34 (1.5)
Marital status	
Single	70 (21)
Married	151 (45.3)
Divorced	54 (16.2)
Widowed	58 (17.4)

(Continued)

Table I (Continued).

Variables	Frequency and Percentile Number (%)
Education level	
No formal education	112 (33.6)
Can read and write	49 (14.7)
Primary education	87 (26.1)
Secondary education	61 (18.3)
Higher Education	24 (7.2)
Occupation	
House wife	85 (25.5)
Employee	54 (16.2)
Student	54 (16.2)
Merchant	76 (22.8)
Daily laborer	36 (10.8)
Farmer	16 (4.8)
Others	12 (3.6)
Residence	
Urban	195 (58.6)
Rural	138 (41.4)

Clinical Characteristics

Before initiation of HAART, approximately 151 (45.3%) of the study participants were in WHO clinical stage I, while the rest of the patients were 95 (28.5%), 61 (18.3%), and 26 (7.8%) were in WHO clinical stages II, III, and IV, respectively. However, after taking HAART for at least six months, almost all of the study participants were in WHO clinical stage I, 330 (99.1%), the remaining 3 patients (0.9%) were in Stage II. The results revealed an increase in the mean BMI of the study participants; 20.14 before starting HAART and 22.19 after taking HAART for at least six months. It was found that 271 (81.6%) participants had no history of opportunistic infections. Of these patients, 31 (50%) had pulmonary tuberculosis, 18 (29.04%) had severe bacterial pneumonia, and 13 (20.96%) had extra pulmonary tuberculosis. Functional status at base line indicated that 278 (83.5%) were working patients. Treatment interruptions were 114 (34.2%) at different points of follow-up (Table 2).

Hematological and Immunological Values Before and After at Least Six Months of HAART

WBC has changed from 4.106 ± 0.970 to 4.995 ± 1.34 ($10^9/l$); there was an increase in Hgb concentration from 12.38 ± 1.77 to 13.22 ± 1.81 (g/dl); MCV has changed from 87.57 ± 11.63 to 89.99 ± 8.87 (fl); also platelet shows change from 243.3 ± 52.59 to 292.18 ± 60.539 ($10^9/l$) and CD4 changed from 356.56 ± 212.32 to 583.7 ± 238.25 cells/mm³ resulting in a mean change of 227.14 cells/ μ L.

The study revealed a significant change in hematological parameters (WBC, Hgb, MCV, and CD4) after the use of HAART for at least six months of initiation of HAART ($P = 0.0001$), with the exception of MCHC ($p = 0.706$) (Table 3).

Hematological Abnormalities Before and After at Least Six Months of HAART

According to WHO classification of anemia based on Hgb concentration, 158 (47.4%) were anemic at baseline, and the magnitude decreased to 77 (23.1%) after at least 6 months of HAART initiation. When data were analyzed by severity, at base line of the 158 anemic study participants majority of them had mild 142 (89.9%) anemia, while 10 (6.3%) moderate, and 6(3.8%) severe. However, after taking HAART for at least six months, while the prevalence of anemia decreased from 47.4% to 23.1%, the severity remained almost same where 71 (92.2%), 2(2.6%),4 (5.2%) had mild, moderate, and severe anemia, respectively (Table 4).

Table 2 Clinical Characteristics of Adult HIV Patients Taking HAART for at Least Months at Yabelo General Hospital, Borana, Ethiopia, 2021

Variables	Frequency and Percentile Number (%)
Initial WHO Clinical stage	
Stage I	151 (45.3)
Stage II	95 (28.5)
Stage III	61 (18.3)
Stage IV	26 (7.8)
Current WHO clinical Stage	
Stage I	330 (99.1)
Stage II	3 (0.9)
Initial BMI	
18.5–24.99	269 (80.8)
<18.49	54 (16.2)
>25	10 (3)
Current BMI	
18.5–24.99	225 (67.6)
<18.49	52 (15.6)
>25	56 (16.8)
Treatment interruption	
Yes	114 (34.2)
No	219 (65.8)
Functional status at base line	
Working	278 (83.5)
Ambulatory	41 (12.3)
Bedridden	14 (4.2)
History of opportunistic infection	
Yes	62 (18.6)
No	271 (81.4)
Type of opportunistic infection	
Severe bacterial pneumonia	18 (29.04)
Pulmonary tuberculosis	31 (50.0)
Extra pulmonary tuberculosis	13 (20.96)

Other hematological abnormalities like leukopenia (WBC < 4000cells/ μ L), Neutropenia (Neutrophil < 1500cells/ μ L; <40%), lymphopenia (lymphocyte <1000cells/ μ L; <20%) and thrombocytopenia (PLT < 150,000cells/ μ L) has shown changes in prevalence between baseline and at least 6 months after therapy. Accordingly rates of leukopenia is reduced from 244 (73.3%) to 121 (36.3%), lymphopenia from 256 (76.9%) to 119 (35.7%), and thrombocytopenia from 11 (3.3%) to 8(2.4%). In contrast, Neutropenia increased from 78 (23.4%) to 194 (58.3%) (Table 4).

Baseline Immunosuppression Level by Age Category in HIV Patients Before and After at Least Six Months of HAART

Of the total study participants, 207 (62.2%) had immune suppression (decrements in CD4 count < 350 cells/ μ L) at initiation of HAART, of which 95 (45.9%) had severe immune suppression as defined by CD4 < 200 cells/ μ L. There was also increment in CD4 count after initiation of HAART, which revealed a decreased immune suppression 69 (20.7%) while severe immunodeficiency decreased from 45.9% to 20.7% (Table 4).

Table 3 Hematological and Immunological Parameters of Adult HIV-Positive Individuals Before and After Initiation of HAAART at Yabelo General Hospital, South Ethiopia, 2021

Parameters	Before Initiation of HAART (n= 333) Mean $\pm$ SD	After Six Months of HAART Initiation (n= 333) Mean $\pm$ SD	t-value (95% *CI)	P-value
WBC ($\times 10^9$ /l)	4.106 $\pm$ 0.970	4.995 $\pm$ 1.34	-12.186 (-1.03277-(-0.74567))	0.0001
LC ($\times 10^9$ /l)	1.097 $\pm$ 0.589	1.588 $\pm$ 0.70	-11.637 (-574,007-(-40,801))	0.0001
ANC ($\times 10^9$ /l)	2.180 $\pm$ 1.212	1.540 $\pm$ 0.86	-10.536 (0.52105-0.76027)	0.0001
RBC ($\times 10^{12}$ /l)	3.929 $\pm$ 0.835	4.482 $\pm$ 0.65	-10.805 (-0.65432-(-45,277))	0.0001
Hgb (g/dl)	12.38 $\pm$ 1.77	13.22 $\pm$ 1.81	-6.926 (-1.09739-(-0.61192))	0.0001
HCT (%)	36.372 $\pm$ 5.60	39.92 $\pm$ 6.32	-8.775 (-4.34832-(-2.7557))	0.0001
MCV (fl)	87.57 $\pm$ 11.63	89.99 $\pm$ 8.87	-4.054 (-3.59256-(-1.24522))	0.0001
MCH (pg)	29.24 $\pm$ 3.67	31.33 $\pm$ 4.89	-6.16 (-2.74833-(-1.41791))	0.001
MCHC (g/l)	31.99 $\pm$ 2.84	32.06 $\pm$ 2.22	-0.378 (-43,077-(0.29191))	0.706
PLT ($\times 10^9$ /l)	243.3 $\pm$ 52.59	292.18 $\pm$ 60.539	-14.597 (-55.467-(-42.293))	0.0001
CD4 (Cells/mm3)	356.56 $\pm$ 212.32	583.7 $\pm$ 238.25	-16.613 (-254.04-(-200.247))	0.0001

Table 4 Prevalence of Immunohematological Abnormalities of Adult HIV Patients Taking HAART for at Least 6 Months at Yabelo General Hospital Borana, Ethiopia, 2021

Hematological Disorder	Before Initiation of HAART Number (%)	After 6 Months of Initiation of HAART Number (%)
Anemia	158(47.4)	77 (23.1)
Mild	142(89.9)	71(92.2)
Moderate	10(6.3)	2(2.6)
Severe	6(3.8)	4(5.2)
Anemia		
Male	78(50)	32(43.7)
Female	80(50)	45(56.3)
Leucopenia	244(73.3)	121(36.3)
Male	132(54.1)	55(45.5)
Female	112(45.9)	66(54.5)
Neutropenia	78(23.4)	194(58.3)
Male	43(55.1)	103(53.1)
Female	35(44.9)	91(46.9)
Lymphopenia	256(76.9)	119(35.7)
Male	136(53.1)	58(48.7)
Female	120(46.9)	61(37.4)
Thrombocytopenia	11(3.3)	8(2.4)
Male	4(36.4)	5(62.5)
Female	7(63.6)	3(37.5)
Immunodeficiency	207(62.2%)	69(20.7%)
Severe	95(45.9%)	18(26.1%)
Mild	112(54.1%)	51(73.9%)

The majority of the study participants had a CD4 count of <350 cells/ μL , regardless of age category. Specifically, 12.9%, 11.4%, 6.9% of the participants in the age groups 29–38, 18–28, and 39–48 years, respectively, were in the CD4 count 200–349 cells/ μL category. Severe immunodeficiency (CD4 < 200 cells/ μL) was predominant in the older age groups of 49–58 and >59 years.

In contrast to the baseline data, the majority of the participants after taking HAART for at least 6 months were found in the CD4 > 500 cells/ μL category, irrespective of age category, although the highest proportion was observed in the age group 18–28 years where 71.3% were in the CD4 > 500 cells/ μL category. The proportion of patients with severe immunosuppression of CD4 < 200 cells/ μL increased with increasing age category which is 12.3%, 21.7%, 24.5%, 33.3%, and 40.0% for the age groups 18–28, 29–38, 39–48, 49–58 and >59 years, respectively (Figure 1).

Factors Associated with Anemia

We further analyzed factors associated with current anemia using the logistic regression model. Those variables with $P \leq 0.20$ in the bivariate analysis were used as a cutoff value to determine candidates for multivariable logistic regression. Based on the above assumption, treatment interruption, type of opportunistic infection, BMI, sex, WHO clinical staging, residence, and educational status were candidate variables for multivariable analysis. Among the variables included in the final model, sex and treatment interruption were associated with current anemia. Those individuals with BMI less than 18.49 are more likely to develop anemia (5.4 times) [OR=5.428] 95% CI, 1.425–20.674] when compared to those with normal BMI, and female patients were more likely to have anemia when compared to male patients [OR=2.02 (95% CI, 1.164–3.503]. Besides, those patients with treatment interruption were more likely to be anemic than those that do not [OR=1.835 (95% CI, 1.003–3.359)] (Table 5).

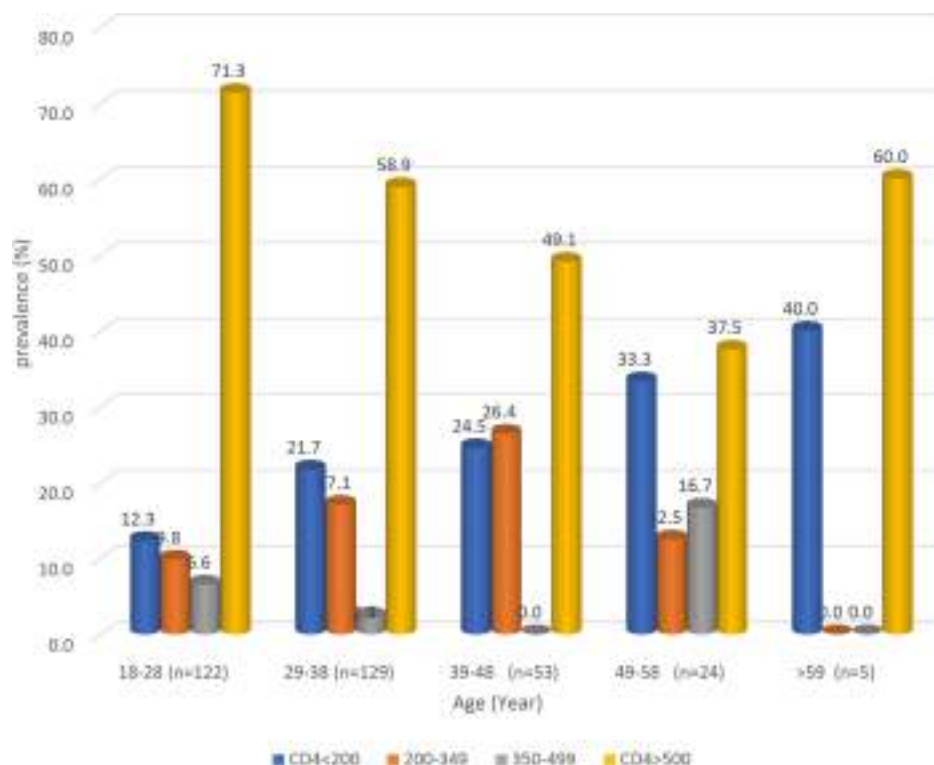


Figure 1 Prevalence of immunosuppressed Adult HIV patients with age category that had been taking HAART for at least 6 months at Yabelo General Hospital, Borana, Ethiopia, 2020.

Table 5 Factors Associated with Anemia After Taking HAART for at Least Six Month at Yabelo General Hospital, South Ethiopia, 2021

Variables	Anemia		COR (95% CI)	P -value	AOR(95% CI)	P-value
	Yes Number (%)	NO Number (%)				
Treatment interruption						
Yes	19(16.7)	95(83.3)	0.555(0.312–0.988)	0.046*	0.545(0.298–0.997)	0.049*
No	58(26.5)	161(73.5)	1		1	
Types of opportunistic						
Severe bacterial pneumonia	5(27.8)	13(72.2)	1.270(0.436–3.699)	0.661	1.454(0.478–4.424)	0.510
Pulmonary tuberculosis	3(9.7)	28(90.3)	0.354(0.104–1.202)	0.096	0.356(0.101–1.247)	0.106
Extra pulmonary tuberculosis	6(46.2)	7(53.8)	2.830(0.918–8.728)	0.070	2.988(0.937–9.533)	0.064
No opportunistic infection	63(23.2)	208(76.8)	1		1	
Current BMI						
18.5–24.9	61(22.7)	208(77.3)	1		1	
<18.49	5(50)	5(50)	3.410(0.956–12.166)	0.059	5.428(1.425–20.674)	0.013*
>24.99	11(20.4)	43(79.6)	0.872(0.424–1.794)	0.710	0.955(0.452–2.030)	0.905
Age						
18–28	26(21.3)	96(78.7)	1			
29–38	31(24)	98(76)	0.856(0.473–1.548)	0.607		
39–48	11(14.3)	42(79.2)	1.034(0.468–2.285)	0.934		
>49	9(31)	20(69)	0.602(0.245–1.478)	0.268		
Sex						
Male	32(18.8)	138(81.2)	1		1	
Female	45(27.6)	118(72.4)	1.646(0.982–2.754)	0.059	2.02(1.164–3.503)	0.012*
WHO stage Current						
Stage 1	75(23)	251(76.9)	0.747(0.142–3.929)			
Stage 2	02(28.6)	5(71.4)	1	0.731		
CD4 at current						
350–499	12(18.2)	54(81.8)	1			
<349	17(25.8)	49(74.2)	1.561(0.678–3.595)	0.295		
>500	48(23.9)	153(76.1)	1.106(0.583–2.097)	0.758		
Residence						
Urban	48(24.6)	147(75.4)	1			
Rural	29(21)	109(79)	1.227(0.727–2.071)	0.443		
Educational Status						
No formal education	30(26.8)	82(73.2)	0.911(0.33–2.512)			
Can read and write	11(22.4)	38(77.6)	1.152(0.368–3.607)	0.857		
Primary education	14(16.1)	73(83.9)	1.738(0.586–5.152)	0.809		
Secondary	16(26.2)	45(73.8)	0.938(0.317–2.777)	0.319		
Higher education	6(25)	18(75)	1	0.907		
Occupation						
House wife	17(20)	68(80)	0.8(0.16–3.996)			
Employee	15(22.4)	39(72.2)	1	0.786		
Student	7(13)	47(87)	1.343(0.242–7.449)	0.736		
Merchant	20(26.3)	56(73.7)	0.56(0.113–2.779)	0.478		
Daily labor	10(27.8)	26(72.2)	0.52(0.097–2.802)	0.238		
Farmer	8(28.6)	20(71.4)	0.333(0.054–2.067)	0.038*		

Note: *Statistically Significant.

Discussion

Our study investigated immunohematological changes among HIV-positive adults after initiation of HAART for more than six months at the Yabelo General Hospital. The study revealed a significant change in hematological parameters after the use of HAART for at least six months, with the exception of MCHC. In addition, there was an average increase of 227.14 CD4 cells/ μ L after treatment. The most common abnormalities include anemia, thrombocytopenia, leukopenia, lymphopenia, immunosuppression, and neutropenia. Low CD4 count is the most important immunological abnormality. Studies have revealed that HAART for at least six month has decreased the prevalence of both hematological and immunological abnormalities.^{12–24}

Our finding of anemia at baseline is in line with a study conducted in Addis Ababa, Ethiopia, by Woldeamanuel et al in 2018 at Black Lion Specialized Hospital (41.9%) and Assefa et al in 2015 at Zewditu Memorial Hospital (42.9%)^{22,25} indicating the effect of HIV infection in the country. The prevalence of anemia before HAART initiation in this study was higher than other studies conducted in other parts of Ethiopia in 2014 by Enawgaw et al (29.7%), Tsegaye et al in 2018 at Ras Desta Memorial Hospital (24.1%), Daka et al in 2013 at Hawassa University Referral Hospital (23.4%), Damtie et al in 2021 at Debre Tabor Comprehensive Specialized Hospital (31.8%), and Uguma et al in 2021 at Goba referral Hospital (37.1%).^{10,13,26–28} This discrepancy might be due to the differences in life style of ART patients, the presence of opportunistic infections, and nutritional status of the study participants in the other studies.

The baseline prevalence of anemia in our study was lower than the prevalence reported in 2013 by Denu et al in Nigeria (57.5%), Owiredo et al in Ghana with 63% before HAART, and Saha et al with 46% after HAART in 2015 in India.^{18,29,30} This discrepancy might be attributed to the differences in the life style, study population, sample size, socio-demographic characteristics of study subjects, and variability in the definition of anemia. Relating to the prevalence of anemia after receiving HAART for at least six months, our findings were 23.1%. This result is in agreement with study conducted in Addis Ababa (20.9%), Debre Tabor (17.4%), and Nigeria (24.3%).^{23,25,29} This prevalence is higher than that reported in other parts of Ethiopia, such as Gondar (11.7%), Addis Ababa (11.4%), and Goba (14.6%).^{4,22,28} This difference in prevalence may be attributed due to life style, altitude, study population, sample size, sociodemographic characteristics of study subjects.

In the current study, the prevalence of anemia after receiving HAART for at least six months was significantly associated with treatment interruption, sex, and BMI greater than 18.99. Female patients were more likely to have anemia when compared to male patients which is supported by study done in Dessie, Ethiopia.²⁸ The result of this study has shown that leucopenia was the most common hematological abnormality with higher prevalence at before Starting of HAART than after taking HAART for at least 6 months (73.3% vs 36.35%), this finding is consistent with the reports of various studies affirming the knowledge that the HIV attacks white blood cells of patients.^{22,29,30}

In the current study, the increase in mean WBC count after six months of HAART initiation indicated boosting of the immune system (4.106 ± 0.970 to 4.995 ± 1.34). The finding was supported, by study in Makurdi, Benue state of Nigeria which indicated that HAART brings statistically significant increment in WBC from $4.07 \pm 0.25 \times 10^3$ / μ L at baseline to $4.76 \pm 0.15 \times 10^3$ / μ L after ART.²⁹ The increase in WBC count after HAART initiation might be due to an increase in hematopoietic progenitor cell growth following a decrease in HIV viral load.²⁶

In contrast with the current findings, other reports have shown a decrease in WBC count after HAART.²⁷ This difference may be attributed to the short duration of HAART intake in other studies, as in the initial stage of ART initiation; WBC count will be low, which gradually adjusts itself over time.

The prevalence of Neutropenia In the present study was 23.4% before HAART initiation and 58.3% after taking HAART for at least six months. This finding contradicts with reports from Gondar (14.8%) and South Korea (10%) at base line and 28.3% in Gondar⁵ and 8.9% in South Korea⁸ after initiation of HAART. The higher prevalence of neutropenia might be attributed to opportunistic and other infections observed in our study.

The present study also revealed differences in mean CD4+ T cell counts before and after HAART initiation (356.56 ± 212.32 vs 583.7 ± 238.25 cells/ μ L), with an average increase of 227.14 CD4 cells/ μ L following treatment. In contrast, previous studies conducted at Zewditu Memorial Hospital reported limited increments after HAART (116 ± 69.4 vs 112 ± 67 cells/ μ L).⁶ This can be explained by the policy change since the research was conducted with regard to ART initiation,

where treatment is administered regardless of CD4 count in recent years, so that the participants of the current study had better initial CD4 counts compared to the earlier studies.

The lower CD4 count at baseline is due to the HIV infection, which decreases the production of CD4⁺ T cells by preventing the maturation of lymphoid precursors. Inhibition of viral replication by HAART is therefore associated with improved production of CD4⁺ T cells.^{4,17,19,20} Highly active antiviral treatment decreases the plasma viral load after taking the medication for at least six months which then increases the CD4 count.⁵

The prevalence of thrombocytopenia in the present study was high at baseline compared to that in patients on HAART for at least six months. Thrombocytopenia was reduced from 3.3 to 2.4% after at least six months of HAART. This result is consistent with the findings of a study in India.¹⁸ The improvement in thrombocytopenia might be attributed to the effect of treatment, in which after HAART initiation, abnormalities of hematopoietic, opportunistic infections, and immune causes related to HIV leading to low platelet count could be reverted.^{27,29}

Also, research in Gondar (9% to 4.1%) and Bale (Goba Referral Hospital) (11.4% to 4.5%) has revealed decrease in thrombocytopenia after they took HAART for at least six months, but the results were slightly higher than the current findings.^{4,28} The variations in the results observed in this study were attributed to variations in the definition of thrombocytopenia, size of the study population, and study design used.

In summary, this study was conducted with the objective of identifying changes in Immuno-hematological parameters among adult HIV-positive patients receiving HAART for at least six months in Borana, Yabelo General Hospital. As shown in these studies, participants at baseline had significantly higher rates of anemia, thrombocytopenia, and CD4⁺ T lymphocytopenia, which improved dramatically after HAART initiation. The finding of a significant number of immunodeficiency, 207 (62.2%) (CD4 count < 350 cells/ μ L) at initiation of HAART, and severe immunodeficiency in 95 (45.9%) of them as defined by CD4 < 200 cells/ μ L indicated quite large number of individuals were living with the virus in the study area. This needs critical attention of the local health authorities to encourage people to get HIV tested so that the benefit of HAART can be maximized as it is given to those tested positive irrespective of CD4 count.

Conclusion

This study showed that the most common hematologic abnormalities (outcomes) in HIV/AIDS patients were anemia, thrombocytopenia, leucopenia, lymphopenia, neutropenia, and thrombocytopenia. Anemia after HAART initiation is linked to a number of factors, including treatment interruption, current BMI and sex. In order for infected patients to benefit from early commencement of HAART, it is necessary to promote HIV testing and early HAART initiation in the pastoralist population due to the observation of a significant percentage of immunosuppressed people at baseline.

Abbreviations

AIDS, Acquired immune deficiency syndrome; AOR, Adjusted odds ratio; ART, Antiretroviral therapy; BMI, Body Mass Index; CBC, Complete blood count; CD4, Cluster of differentiation 4; HAART, Highly active antiretroviral treatment; Hgb, Hemoglobin; HIV, Human immunodeficiency virus; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; OI, Opportunistic infection; OR, Odds ratio; PLT, Platelets; RBC, Red blood cell; SD, Standard Deviation; SPSS, Statistical Package for Social Sciences; WBC, White blood cell; YGH, Yabelo general hospital; WHO, World health organization.

Acknowledgments

We would like to thank Addis Ababa University, the study participants and Yabelo General Hospital staff, especially the data collectors and laboratory staff, for their valuable contributions.

Disclosure

The authors declare no conflicts of interest in this work. This paper is based on the thesis by Girma Ashenafi. It has been published on the institutional website: <http://etd.aau.edu.et/handle/123456789/28526>.

References

- Patwardhan M, Golwilkar A, Abhyankar JR, Atre M. Hematological profile of HIV positive patients. *Indian J Pathol Microbiol.* 2002;45(2):147-150.
- Clark S. Experts predict global devastation due to HIV/AIDS. *Lancet.* 2002;360(9327):145. doi:10.1016/S0140-6736(02)09439-4
- Stover J, Walker N, Garnett G. Can we reverse the HIV/AIDS pandemic with an expanded response. *Lancet.* 2002;360(9326):373-377.
- Geletaw T, Tadesse MZ, Demisse AG. Hematologic abnormalities and associated factors among HIV infected children pre- and post-antiretroviral treatment, North West Ethiopia. *J Blood Med.* 2017;8(8):99-105. doi:10.2147/JBM.S137067
- World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. World Health Organization; 2007.
- Mathews S, Bala DS, Sharma A. Association of hematological profile of human immunodeficiency virus-positive patients with clinicoimmunologic stages of the disease. *J Lab Physicians.* 2013;5(1):34-37. doi:10.4103/0974-2727.115929
- Gifford RJ, De-oliveira T, Rambaut A. UK collaborative group on HIV drug resistance: phylogenetic surveillance of viral genetic diversity and the evolving molecular epidemiology of human immunodeficiency virus type 1. *J Virol.* 2007;81(23):13050-13056. doi:10.1128/JVI.00889-07
- UNAIDS. AIDS epidemic Gap report; 2020.
- Choi SY, Kim I, Kim NJ, Lee S, Choi Y, Bae J. Hematological manifestations of human immunodeficiency virus infection and the effect of highly active antiretroviral therapy on cytopenia. *Korean J Hematol.* 2011;46(4):253-257. doi:10.5045/kjh.2011.46.4.253
- Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar. *BMC Hematol.* 2014;14(1):14-18. doi:10.1186/2052-1839-14-14
- Lu DY, Wu HY, Yarla NS, Xu B, Ding J, Lu TR. HAART in HIV/AIDS Treatments: future Trends. *Infect Disord Drug Targets.* 2018;18(1):15-22. doi:10.2174/1871526517666170505122800
- Derbe M, Monga D, Daka D. Immunological response among HIV/AIDS patients before and after ART therapy at Zewuditu Hospital Addis Ababa, Ethiopia. *Am J Res Commun.* 2013;1(1):103-115.
- Tsegaye A, Desta K, Oma D, et al. Prevalence of anemia before and after initiation of antiretroviral therapy on HIV infected patients at Ras Desta Damtew Memorial Hospital, Addis Ababa, Ethiopia. *RR/MHS.* 2018;7(4):24-30.
- HIV status in Ethiopia. UNAIDS; 2018. Available from: <https://www.unaids.org/en/regionscountries/countries/ethiopia>. Accessed January, 2021.
- Huruy K, Kassu A, Mulu A, Wondie Y. Immune restoration disease and changes in CD4+ T cell count in HIV-infected patients during highly active antiretroviral therapy at Zewditu memorial hospital, Addis Ababa, Ethiopia. *AIDS Res Ther.* 2010;7(1):1-7. doi:10.1186/1742-6405-7-46
- Adane A, Desta K, Bezabih A, Gashaye A, Kassa D. HIV-associated anemia before and after initiation of antiretroviral therapy at ART Centre of Minilik II Hospital, Addis Ababa, Ethiopia. *Ethiop Med J.* 2012;50(1):13-21.
- Gedefaw L, Yemane T, Sahlemariam Z, Yilma D. Anemia and risk factors in HAART naïve and HAART experienced HIV positive persons in south west Ethiopia: a comparative study. *PLoS One.* 2013;8(8):e72202. doi:10.1371/journal.pone.0072202
- Saha D, Kini J, Subramaniam R. A study of the hematological profile of human immunodeficiency virus positive patients in coastal Indian region. *Med sci.* 2015;35(5):190-193.
- Qazi RA, Bashir N, Daud Y. Correlation of CD4 lymphocyte count with haemoglobin concentration in HIV infected patients at HIV treatment centre. *Med sci.* 2013;9(3):138-140.
- Robinson O, Obianime AW, Tamun I. Immunological and Hematological profile of HIV patients on antiretroviral therapy in port harcourt, River state Nigeria. *Int Blood Res.* 2017;7(4):1-12.
- Addis Z, Yitagezu G, Tacheble B. prevalence of some hematological abnormalities among HIV positive patients on their first visit to tertiary health institution. *Int Blood Res.* 2014;2(6):270-277. doi:10.9734/IBRR/2014/11137
- Woldeamanuel G, Wondimu H. Prevalence of anaemia before and after initiation of antiretroviral therapy among HIV infected Patients at black lion specialized hospital Addis Ababa, Ethiopia. *BMC Hematol.* 2018;18:7. doi:10.1186/s12878-018-0099-y
- Fenetahun Y, Fentahun T. Socio-economic profile of arid and semi-arid agro-pastoral region of Borana rangeland Southern Ethiopia. *MOJ Eco Environ Sci.* 2020;5(3):113-122.
- Negesse A, Getaneh T, Temesgen H. Prevalence of anemia and its associated factors in human immuno deficiency virus infected adult individuals in Ethiopia. A systematic review and meta-analysis. *BMC Hematol.* 2018;8:32. doi:10.1186/s12878-018-0127-y
- Assefa M, Abegaz WE, Shewamare A, Medhin G, Belay M. Prevalence and correlates of anemia among HIV infected patients on highly active antiretroviral therapy at Zewditu Memorial Hospital, Ethiopia. *BMC Hematol.* 2015;15(1):1-6. doi:10.1186/s12878-015-0024-6
- Daka D, Lelissa D, Amsalu A. Prevalence of anemia before and after the initiation of antiretroviral therapy at ART center of Hawassa University Referral Hospital, Hawassa, South Ethiopia. *Sch J Med.* 2013;3(1):1-6.
- Damtie S, Lemma W, Teklehaimanot K, Tahir E, Tegenaw T. Hematological abnormalities of adult HIV-infected patients before and after initiation of highly active antiretroviral treatment at Debre Tabor Comprehensive Specialized Hospital, Northcentral Ethiopia: a Cross-Sectional Study. *HIV/AIDS Res Palliat Care.* 2021;4(13):477-484.
- Uguma N, Kiya GT, Maleko WA, Bimerew LG. Hematological parameters abnormalities and associated factors in HIV-positive adults before and after highly active antiretroviral treatment in Goba Referral Hospital, South-East Ethiopia. *SAGE Open Med.* 2021;9:1-12.
- Denué BA, Kida IM, Hammagabdo A, Dayar A, Sahabi MA. Prevalence of anemia and immunological markers in HIV-infected patients on highly active antiretroviral therapy in Northeastern Nigeria. *Infect Dis Res Treat.* 2013;6:25-33.
- Owiredun WK, Quayle L, Amidu N, Addai-Mensah O. Prevalence of anaemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. *Afr Health Sci.* 2011;11(1):2-15.

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



The Correlation Between sP-Selectin and Platelet Count in COVID-19 Patients in Referral Hospital, West Java Indonesia

Delita Prihatni, Frany Charisma Budianto, Basti Andriyoko & Suryarini Trisa

To cite this article: Delita Prihatni, Frany Charisma Budianto, Basti Andriyoko & Suryarini Trisa (2023) The Correlation Between sP-Selectin and Platelet Count in COVID-19 Patients in Referral Hospital, West Java Indonesia, Journal of Blood Medicine, , 555-561, DOI: [10.2147/JBM.S425667](https://doi.org/10.2147/JBM.S425667)

To link to this article: <https://doi.org/10.2147/JBM.S425667>



© 2023 Prihatni et al.



Published online: 27 Oct 2023.



Submit your article to this journal



Article views: 138



View related articles



View Crossmark data

The Correlation Between sP-Selectin and Platelet Count in COVID-19 Patients in Referral Hospital, West Java Indonesia

Delita Prihatni¹, Frany Charisma Budianto¹, Basti Andriyoko¹, Suryarini Trisa²

¹Clinical Pathology Department, Medical Faculty of Padjadjaran University, Hasan Sadikin Hospital, Bandung, West Java, Indonesia; ²Depati Hamzah Hospital, Bangka Belitung, Indonesia

Correspondence: Delita Prihatni, Hasan Sadikin Hospital, Jln. Pasteur No. 38, Bandung, West Java, Indonesia, Tel +628122314089, Email delita.prihatni@unpad.ac.id

Introduction: sP-selectin is a glycoprotein located in α granules of platelet and endothelial's Weibel Palade body, as expression to platelet activation and endothelial cell stimulation by SARS-CoV-2 binding with ACE2 receptor. Consumptive thrombocytopenia is also related to platelet activation. Elevation of sP-selectin and thrombocytopenia are related to COVID-19 complication and often correlated with severity of COVID-19.

Purpose: Assess the correlation between sP-selectin and platelet in COVID-19 patients at intensive care and non-intensive care.

Patients and Methods: The study population was hospitalized COVID-19 patients confirmed by Real-Time PCR that underwent platelet examination within 48 hours upon admission, divided into intensive care and non-intensive care group. sP-selectin examination using ELISA methods. Platelet cell count and sP-selectin divided based on normal reference range.

Results: The subjects consist of 24 were in intensive care, 25 were in non-intensive care group. A 66.7% of subject in intensive care group has an elevation in sP-selection (>44.0 ng/mL). Thrombocytopenia was significantly correlated with intensive group ($r = -0.32$, $p < 0.05$). The combination of platelet count $<150.000/\text{mm}^3$ and sP-selectin $>44.0\text{ng/mL}$ was not correlated with the intensive and non-intensive group. Platelet and sP-selectin were not correlated with each other.

Conclusion: Thrombocytopenia is able to induce the expression of sP-selectin.

Keywords: COVID-19, sP-selectin, platelet, thrombocytopenia

Introduction

Coronavirus Disease 2019 (COVID-19) is a new infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which was first identified in the city of Wuhan in December 2019. SARS-CoV-2 belongs to the Coronaviridae family and the genus Betacoronavirus. This virus is a positive single-stranded RNA virus that is encapsulated, non-segmented, and spherical in shape with a diameter of 60–140 nanometers. Transmission of this virus can be transmitted from human to human through respiratory droplets and aerosols. In addition, the RNA of this virus has also been found in the feces of people with COVID-19.^{1,2}

SARS-Cov-2 consists of four main structures, namely spike glycoprotein (S), matrix (M), nucleocapsid (N), and capsule (E). Protein S will bind to Angiotensin-converting enzyme 2 (ACE2) as a receptor to enter the host cell after being activated by transmembrane protease serine 2 (TMPRSS2).²

Angiotensin-converting enzyme 2 (ACE2) is a type 1 transmembrane protein with 805 amino acids attached to the outer surface of cells.¹ ACE2 is found mostly on the surface of alveolar epithelial cells and enterocyte cells in the small intestine. The expression of the ACE2 receptor on the surface of the alveoli that causes the clinical symptoms of COVID-19 is generally related to damage to the lung parenchyma.³ ACE2 receptor is also present in the endothelial cells of arteries and veins, as well as in arterial smooth muscle cells, therefore patients with COVID-19 may also experience complications of coagulopathy.⁴

The mechanism of coagulopathy in COVID-19 is not fully understood, but it is thought to be related to the binding of SARS-Cov-2 to ACE2 receptors on endothelial cells. SARS-Cov-2 can directly cause endothelial disease, leading to endothelial dysfunction, endothelial cell lysis and death. Furthermore, activation of neutrophils, formation of pro-inflammatory cytokines and vasoactive molecules due to inflammation can also increase vascular permeability, leading to endothelial cell damage. Endothelial cell damage will expose a thrombogenic basement membrane that activates the coagulation cascade and activates platelets. Pro-inflammatory cytokines (IL-1 β and TNF) exposure in endothelial cell will result in expression of P-selectin, von Willebrand factor and fibrinogen and will lead to further thrombosis and coagulopathy. Furthermore, platelet activation can also increase the expression of P-selectin on the surface of platelets.^{5–12} Therefore, elevated plasma levels of P-selectin in patients with COVID-19 can be used as a marker of endothelial dysfunction and platelet activation and is associated with coagulopathy and disease severity.¹³

Platelet activation in COVID-19 coagulopathy may lead to thrombocytopenia in COVID-19 patients as the severity of COVID-19 patients increases. Thrombocytopenia in patients with COVID-19 was found in several studies, one of them was the study by Liu et al, which found a correlation between platelets and mortality in patients with COVID-19.¹⁴

According to that theory, coagulopathy leads to worsening of COVID-19 may be manifested by increased sP-selectin and thrombocytopenia. SP-selectin was considered as a new and limited parameter in Indonesia, so there are not many studies about the correlation between sP-selectin and platelet counts in COVID-19 patients in Indonesia. Therefore, investigators are interested to study about a correlation between these two parameters in patients with COVID-19 treated in ICU and non-ICU isolation wards.

Materials and Methods

This analytical cross-sectional study that included COVID-19 patients admitted into the in-patient ward (intensive and non-intensive care) was conducted at RSUP Dr. Hasan Sadikin Bandung from August to September 2021. This study used purposive sampling with minimal sample was determined using minimal sample equation for correlation study. Subjects were taken from a population of COVID-19 patients who met the inclusion criteria: (1) adult patients (≥ 18 years) diagnosed with COVID-19 based on real-time reverse transcription PCR (RT-PCR) in nasopharyngeal swabs, (2) platelet and D-Dimer examinations are carried out at the initial admission to the isolation inpatient room, or at least within 48 hours after admission to the COVID isolation inpatient care. The exclusion criteria of this study were the patient died before entering the inpatient room, incomplete and inaccessible medical record data during the study period, subjects with incomplete laboratory data.

The subjects were grouped based on the ward requirements, that is intensive and non-intensive wards. Data regarding the treatment room for COVID-19 patients was obtained by tracing the patient's medical record data. D-dimer level was taken for characteristic data. The D-Dimer parameter was divided into two mortality risk groups based on Zhou's research, which were $>1.0 \mu\text{g/mL}$.¹⁵

Data about platelet counts were taken from the laboratory information system based on the results of the examination using the Sysmex XN-1000 hematology analyzer. Platelet results in the study subjects were then divided into two groups, the first group as abnormal group with thrombocytopenia (platelet count $<150.000/\text{mm}^3$) and the second group if the platelet count was normal or above the reference value ($\geq 150.000/\text{mm}^3$).

Blood samples of COVID-19 patients were collected at the time of hospital admission. Citrate peripheral blood was centrifuged at 1200 rpm for 10 minutes at room temperature to obtain plasma samples. After that, 500 μL of plasma samples were stored at -80°C before analyzed to obtain the quantification of soluble P-selectin performed ELISA assay (eLabscience; detection range: 1.56–100 ng/mL). The reference value for sP-selectin is taken based on the reference value on the insert kit, which is 26–44 ng/mL. The results of the sP-selectin were divided into two groups: group one is considered abnormal if the results of the p-selectin examination were $>44 \text{ ng/mL}$ and group two as normal results if sP-selectin was $\leq 44 \text{ ng/mL}$.

Statistical Analysis

All research data were processed using SPSS version 25.0 and tabulated against the subject's treatment room (ICU/non-ICU). Correlation between sP-selectin, platelet counts, and patient's ward were calculated using Mann–Whitney

U method. Correlation between sP-selectin level and Platelet Counts was performed using Goodman and Kruskal's gamma coefficient, if the results were near to 1 then the correlation is stronger. All analyses were two-sided and a p value smaller than 0.05 was considered statistically significant. The results in this study will then be presented in the form of tables and charts.

Results

There were 49 subjects those met the inclusion criteria. Of the 49 subjects, 24 subjects were treated in the ICU and 25 subjects in the non-ICU isolation room. Characteristics data of the subjects of this study can be seen in Table 1.

Based on Table 1, it was found that the age of subjects who were treated in the ICU was almost evenly distributed, while in non-ICU isolation wards were mostly in the age group of 56–65 years (44.0%). The gender distribution of research subjects was dominated by male (66.7% and 60.0%) in the ICU and non-ICU, respectively. Based on laboratory results, it was found that most of the sP-selectin levels in patients who were treated in the ICU had increased levels of sP-selectin (66.7%), while for the group of subjects treated in non-ICU isolation rooms, most of them had a normal levels of sP-selectin (56.0%). The mean platelet counts in the ICU and non-ICU groups were normal (95.8% and 72.0%) in both subjects group. Meanwhile, D-Dimer levels were elevated for both of subjects group (95.8% for ICU subjects and 80.0% for subjects in non-ICU isolation).

From Table 2, we found that only platelet parameters had a significant relationship between ICU and non-ICU with a weak effect ($r = -0.32$, $p < 0.05$). Meanwhile, for the parameters of sP-selectin and the combination of platelet and sP-selectin parameters did not have a significant correlation.

Based on the analysis using Goodman and Kruskal's Gamma coefficient, it shows that the sP-selectin and platelet counts has no correlation. To be clearer, the result of this correlation's analysis is shown in Figure 1.

Table 1 Characteristics of the Research Subjects

Patient Characteristics	COVID-19 Wards			
	ICU (n=24)		Non ICU (n=25)	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Age (year)				
18–25	2	8.4	1	4.0
26–35	5	20.8	1	4.0
36–45	4	16.7	5	20.0
46–55	5	20.8	1	4.0
56–65	5	20.8	11	44.0
>65	3	12.5	6	24.0
Sex				
Male	16	66.7	15	60.0
Female	8	33.3	10	40.0
sP-selectin (ng/mL)				
>44.0	16	66.7	11	44.0
≤ 44.0	8	33.3	14	56.0
Platelet counts (/mm³)				
<150.000	1	4.2	7	28.0
≥150.000	23	95.8	18	72.0
D-dimer (μg/mL)				
≥1	23	95.8	20	80.0
<1	1	4.2	5	20.0

Notes: Patients are divided into two groups according to their hospital ward (Intensive Care and Non-intensive Care Ward). Data were presented in categorical variables as frequencies and percentages. All reported laboratory data were measured maximal at 48 hours after admission.

Table 2 The Correlation of sP-Selectin and Platelets with COVID-19 ICU Patients and Non-ICU Ward

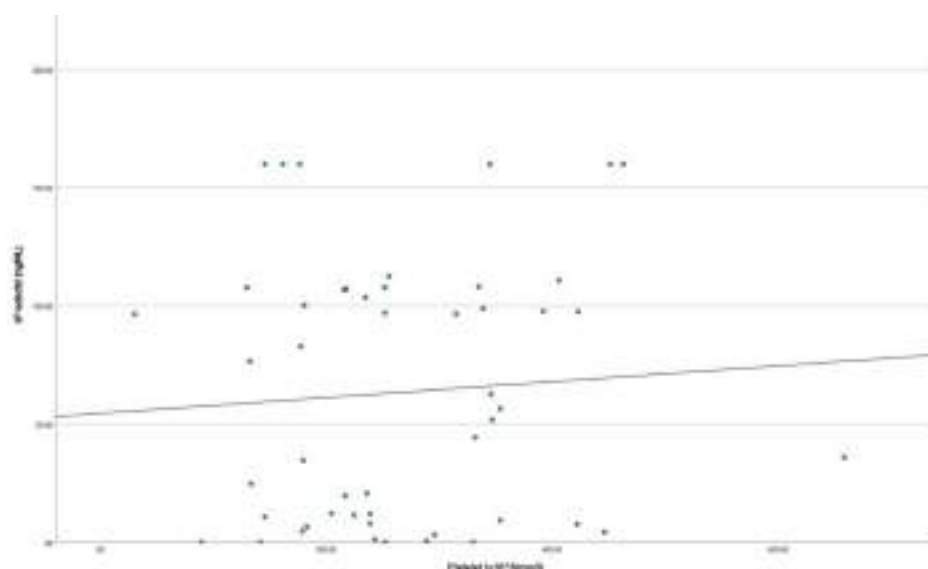
Laboratorium Results	COVID-19 Wards		p-value	r Coefficient (95% CI)
	ICU N (%)	Non-ICU N (%)		
sP-selectin (ng/mL)				
>44.0	16 (32.7%)	11 (22.4%)	0.114	-0.23
≤ 44.0	8 (16.3%)	14 (28.6%)		
Platelet counts (/mm³)				
<150.000	1 (2.1%)	7 (14.3%)	0.026	-0.32
≥150.000	23 (46.9%)	18 (36.7%)		
sP-selectin and platelet counts				
● sP selectin >44.0 and Platelet Count <150.000	1 (4.5%)	3 (13.6%)	0.450	0.153 ^a
● sP selectin ≤44.0 and Platelet Count ≥150.000	8 (36.4%)	10 (45.5%)		

Notes: The laboratorium results were correlated to the patient's hospital ward. Correlation between Soluble P-selectin and hospital ward is analyzed with Mann–Whitney U. Correlation between Platelet Counts and hospital ward is analyzed with Mann–Whitney U with r coefficient nearer to 1.00 were interpreted as stronger correlation. Letter “a” showed significance of combination between abnormal level of sP-selectin and platelet counts to their hospital ward. It was tested using Pearson chi-square test of independence and then followed by Cramer's V test for testing their correlation. Significance of a: if 0-<0.10 negligible, 0.10-<0.30 small / weak correlation, 0.30-<0.50 medium correlation, ≥0.50 large/strong correlation. P value <0.05 were considered significant.

Discussion

SARS-CoV-2 attacks by binding to ACE2 receptors on the surface of endothelial cells, not only in the lungs but also in the blood vessels.⁴ There is one theory that underlies COVID-19 can cause a spectrum of diseases involving the hemostatic system, ie abnormal coagulopathy.^{16,17} Coagulopathy that occurs in COVID-19 patients consists of increased D-dimer, followed by mild thrombocytopenia and finally disseminated intravascular coagulation (DIC).¹⁸

In this study, thrombocytopenia occurred in both subjects treated in the ICU and non-ICU isolation inpatients. Thrombocytopenia in this study had a weak but significant correlation between COVID-19 patients treated in the ICU

**Figure 1** The correlation of sP-selectin with platelet counts.

Notes: Correlation between sP-selectin with platelet counts was analyzed using Goodman and Kruskal's gamma coefficient. If the result is near to 1.00, then the correlation between the two parameters is very strong. X-axis showed the platelet counts result, y-axis for sP-selectin result, the midline showed an ideal correlation. P value <0.05 is statistically significant.

and non-ICU. These results are consistent with the study of Yang et al who found that thrombocytopenia was common in COVID-19 patients with an increased risk of mortality in patients with lower platelets.¹⁹ The results of a meta-analysis by Lippi et al also stated that there was a correlation between thrombocytopenia and the severity of COVID-19.²⁰ The incidence of thrombocytopenia in COVID-19 patients is associated with an increase in platelet consumption due to thrombus formation; however, it usually tends to be mild because it is usually followed by an increase in platelet production. Decreased platelets may indicate worsening of thrombosis and are associated with increased mortality.¹⁸

Based on this study, we found that the levels of sP selectin in patients treated in the ICU mostly increased (66.67%), meanwhile in the non-ICU group mostly had normal levels of sP selectin (56%). These results are following the study by Manne et al which showed an increase in P-selectin levels on the surface of the platelet membrane in COVID-19 patients.²¹ Agrati et al also mentioned a higher plasma P-selectin level in COVID-19 patients compared to healthy controls [median 65.2 (IQRs: 45.1–81.1) vs 40.3 (IQRs: 24.3–48.7), respectively, $p=0.0023$].³

This result was supported by the theory that the expression of P-selectin in COVID-19 is related to damage of endothelial cells leading to coagulation cascade and platelet activation. At normal condition, P selectin is located on the α granules of platelets as well as on the endothelium. Megakaryocytes and endothelial cells produce P-selectin and store it in platelet α granules and Weibel Palade bodies from endothelial cells. In COVID-19, binding of SARS-CoV-2 to ACE2 receptors on the endothelium causes endothelial damage and activates the coagulation cascade and platelet activation.^{5,6} The expression of P-selectin consists of two processes. The first process is when platelet and endothelial cells receive a stimulus in the form of inflammatory mediators such as histamine, thrombin, or a state of hypoxia, they will translocate P-selectin to the surface in the first two minutes and will reach a peak within 10 minutes. This expression will reach normal after three hours. The second process happens when there is stimulation by cytokines that makes P-selectin rise again within 12 hours.^{3,14,22} Therefore, this study shows a higher sP-selectin levels in COVID-19 patients in ICU wards compared to the normal level in non-ICU patients so the sP-selectin level can be considered as the screening of the worsening COVID-19 condition.

When we performed an analytical correlation, this study found no correlation between sP-selectin in ICU-treated COVID-19 patients and non-ICU. This is consistent with the study by Agrati et al, who find no differences in P-selectin levels between ICU-treated COVID-19 patients and in the ICU setting.³

Based on the results of this study, sP-selectin does not correlate with platelet count. According to previous theory, the expression and elevation of sP-selectin levels are associated with platelet activation. Although thrombocytopenia occurs due to platelet activation in coagulation in COVID-19, it is also being balanced by increasing platelet production.¹⁸ In addition, sP-selectin levels are also associated with endothelial stimulation. Therefore, changes in sP-selectin levels are not directly related to changes in platelet count in this study.

This study has several limitations. This study had a small research sample because it was conducted at only one site and was conducted at the end of the COVID Delta period, so the number of incoming patients had decreased. We used no healthy control in this study and using the previous research as the validation of sP-selectin.³ Lopez et al also found that P selectin is less specific for thrombosis than other biomarkers such as von Willebrand Factor, lupus anticoagulant, D-dimer.¹¹ Because of the data limitations in our hospital, we cannot explore more about the platelet activity, either from platelet index or platelet aggregation test and also the comorbidity characteristic is not well mentioned. We suggest to include the platelet index data and platelet aggregation test to see the platelet activation process for further research.

Conclusion

In this study, an increase in sP-selectin levels was found in the group of subjects treated in the COVID-19 ICU, while in the group treated in non-ICU isolation rooms, most of them had normal sP-selectin levels. There is no correlation between sP-selectin levels in COVID-19 patients treated in ICU and non-ICU. Meanwhile, for thrombocytopenia, a significant correlation was found with a weak effect between COVID-19 patients treated in the ICU and non-ICU rooms. While progress has been made in elucidating the molecular mechanisms leading to platelet adhesion, aggregation, shape change and secretion, clinically useful tests of platelet function have been developed. A number of dedicated platelet function instruments have now become available. Some instruments have been incorporated into routine clinical use and can be utilized not only as general screening tests of platelet function but as monitors of antiplatelet therapy and

to potentially assess both risk of bleeding and/or thrombosis.²³ The results of this study are expected to provide information regarding sP-selectin and platelet counts in the development of COVID-19 disease especially in Indonesia as one of the limited resource and developing country.

Ethics Approval and Consent to Participate

The institutional review board of Dr. Hasan Sadikin General Hospital Bandung, Indonesia approved the protocol no LB.02.01/X.6.5/337/2022 and all procedures were in compliance with the Helsinki Declaration. All patients gave their informed consent to participate in the study, which was approved by our local Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung, Indonesia.

Acknowledgment

We thank Mrs. Hijrianti for helping us in preanalytic and analytic sample process of this research, Mulya Nurmansyah for statistical analysis, and Verina Logito for helping us in submitting this research.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Tahmineh Mokhtari FH, Ghaffari N, Ghaffari N, et al. COVID-19 and multiorgan failure: a narrative review on potential mechanisms. *J Mol Histol*. 2020;51(6):613–628. doi:10.1007/s10735-020-09915-3
2. Mohammad Madjid PS-N, Solomon SD, Solomon SD, et al. Potential effects of coronaviruses on the cardiovascular system. *JAMA Cardiol*. 2020;5(7):831–840. doi:10.1001/jamacardio.2020.1286
3. Agrati C, Bordoni V, Sacchi A, et al. Elevated P-selectin in severe Covid-19: considerations for therapeutic options. *Mediterr J Hematol Infect Dis*. 2021;13(1):e2021016. doi:10.4084/mjhid.2021.016
4. Hamming I, Timens W, Bulthuis M, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS Coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004;203(2):631–637. doi:10.1002/path.1570
5. Freedman JE, Lascenzo J. Platelets and fibrinolysis/thrombolysis. *Fibrinolysis in Disease Molecular and Hemovascular Aspects of Fibrinolysis*. CRC; 2019:65–69.
6. Varga Z, Flammer AJ, Steiger P. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417–1418. doi:10.1016/S0140-6736(20)30937-5
7. Lopez-Castaneda S, Garcia-Larragoiti N, Cano-Mendez A, et al. Inflammatory and prothrombotic biomarkers associated with the severity of COVID-19 infection. *Clin Appl Thromb Hemost*. 2021;27:1–9.
8. Tommaso Neri DN, Celi A, Celi A. P-selectin blockade in COVID-19 related ARDS. *Am J Physiol Lung Cell Mol Physiol*. 2020;318(6):L1237–L1238. doi:10.1152/ajplung.00202.2020
9. Alicja Polek WS, Matowicka-Karna J. P-Selectin and Its Role in Some Diseases. *Postepy Hig Med Dosw*. 2009;63:465–470.
10. Corlia Grobler SC, Maphumulo SC, Grobbelaar LM, et al. COVID-19: the Rollercoaster of Fibrin(Ogen), D-Dimer, Von Willebrand Factor, P-selectin and their interactions with endothelial cells, platelets and erythrocytes. *Int J Mol Sci*. 2020;21(14):5168. doi:10.3390/ijms21145168
11. López Reboiro ML, Suárez Fuentetaja R, Gutiérrez López R, et al. Role of lupus anticoagulant and von Willebrand factor in chronic reactive endotheliitis in COVID-19. *J Infect*. 2021;82(6):e27–e28. doi:10.1016/j.jinf.2021.03.006
12. Ming Chen M, Geng J-G. P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis. *Arch Immunol Ther Exp*. 2006;54(2):75–84. doi:10.1007/s00005-006-0010-6
13. Nader Yatim JB, Chocron R, Chocron R. Platelet Activation in Critically Ill COVID-19 Patients. *Ann Intensive Care*. 2021;11(1):113. doi:10.1186/s13613-021-00899-1
14. Liu Y, Sun W, Guo Y, et al. Association between platelet parameters and mortality in coronavirus disease 2019: retrospective cohort study. *Platelets*. 2020;31(4):490–496. doi:10.1080/09537104.2020.1754383
15. Litao Zhang XY, Fan Q, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with COVID-19. *J Thromb Haemost*. 2020;18(6):1324–1329. doi:10.1111/jth.14859
16. Bikdeli B, Madhavan MV, Jimenez D, et al.; Global COVID-19 Thrombosis Collaborative Group, Endorsed by the ISTH, NATF, ESVM, and the IUA, Supported by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2020;75(23):2950–2973. doi:10.1016/j.jacc.2020.04.031
17. Helms J, Tacquard C, Severac F, et al.; CRICS TRIGGERSEP Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med*. 2020;46(6):1089–1098. doi:10.1007/s00134-020-06062-x
18. Wool G, Miller D, J L. The Impact of COVID-19 Disease on Platelets and Coagulation. *Pathobiology*. 2021;88(1):15–27. doi:10.1159/000512007
19. Yang X, Yang Q, Wang Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost*. 2020;18(6):1469–1472. PMID: 32302435. doi:10.1111/jth.14848

20. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin Chim Acta*. 2020;506:145–148. PMID: 32178975; PMCID: PMC7102663. doi:10.1016/j.cca.2020.03.022
21. Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood*. 2020;136(11):1317–1329. doi:10.1182/blood.2020007214
22. Blann AD, Nadar SK, Lip GYH. The adhesion molecule P-selectin and cardiovascular disease. *Eur Heart J*. 2003;24(24):2166–2179. doi:10.1016/j.ehj.2003.08.021
23. Pakala R, Waksman R. Currently available methods for platelet function analysis: advantages and disadvantages. *Cardiovasc Revascular Med*. 2011;12(5):312–322. doi:10.1016/j.carrev.2010.09.005

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Glanzmann Thrombasthenia Associated with Siderotic Synovitis and Arthropathy: A Case Report

Mouhammad J Alawad, Mohammad Abu-Tineh, Awni Alshurafa, Alaa Al-Taie, Anil Yousaf & Mohamed A Yassin

To cite this article: Mouhammad J Alawad, Mohammad Abu-Tineh, Awni Alshurafa, Alaa Al-Taie, Anil Yousaf & Mohamed A Yassin (2023) Glanzmann Thrombasthenia Associated with Siderotic Synovitis and Arthropathy: A Case Report, Journal of Blood Medicine, , 563-567, DOI: [10.2147/JBM.S418937](https://doi.org/10.2147/JBM.S418937)

To link to this article: <https://doi.org/10.2147/JBM.S418937>



© 2023 Alawad et al.



Published online: 02 Nov 2023.



Submit your article to this journal



Article views: 141



View related articles



View Crossmark data

Glanzmann Thrombasthenia Associated with Siderotic Synovitis and Arthropathy: A Case Report

Mouhammad J Alawad¹, Mohammad Abu-Tineh², Awni Alshurafa², Alaa Al-Taie³, Anil Yousaf², Mohamed A Yassin²

¹Department of Medical Education, Internal Medicine Residency Program, Hamad Medical Corporation, Doha, Qatar; ²Department of Medical Oncology, Hematology and BMT Section, National Center for Hamad Medical Corporation, Doha, Qatar; ³Clinical Imaging Department, Hamad Medical Corporation, Doha, Qatar

Correspondence: Mouhammad J Alawad, Department of Medical Education, Internal Medicine Residency Program, Hamad Medical Corporation, PO Box 3050, Doha, Qatar, Tel +974 33078745, Fax +97444397857, Email www.wad7a123@gmail.com

Abstract: Glanzmann thrombasthenia is a bleeding disorder with a low incidence. It typically manifests as superficial bleeding episodes, which tend to be mild. Deep organ involvement is not uncommon but remains rare due to the rarity of the disease itself and the unusual association between platelet disorders and deep organ implications. A 17-year-old boy with Glanzmann thrombasthenia since infancy developed ankle pain after a minor trauma. His initial workup was negative, but he continued to experience ankle pain. A magnetic resonance imaging (MRI) done after four weeks suggested siderotic synovitis. The patient was lost to follow-up after that and returned after two years with recurrent left ankle pain. Imaging and studies have shown evidence of chronic arthropathy. A specialized orthopedic team assessed the patient. The patient underwent intra-articular steroid injection for pain relief and was referred to continue physical therapy. In conclusion, hemarthrosis is more common in hemophilia than in platelet disorders and has potential morbidity and quality-of-life implications.

Keywords: Glanzmann thrombasthenia, hemarthrosis, synovitis

Introduction

Homeostasis is the process of blood clot formation at the site of a vessel injury. It consists of two distinct phases: formation of a platelet plug and clotting cascade activation, which leads to plug stabilization.¹ Platelet activation is an important step that involves cell adhesion, aggregation, and secretion. Platelet aggregation is mediated by exposure to structural changes in the integrin alpha-IIb/beta-3 (α IIb β 3) receptor on the platelet surface, leading to the binding of von Willebrand Factor (VWF) and fibrinogen, resulting in platelet-platelet aggregation.^{1,2}

Glanzmann thrombasthenia (GT) is a rare bleeding disorder caused by a mutant GPIIb/IIIa. It is inherited in an autosomal recessive fashion with an incidence of 1 in 1,000,000 patients.³ GT is mainly characterized by mucocutaneous bleeds, manifested as purpura, epistaxis, gingival hemorrhages, and menorrhagia. Deep organ involvement is well documented but remains sparse in the literature.⁴

Hemarthrosis is an infrequent and unusual condition associated with GT. However, it is a very common manifestation of hemophilia, with most affected patients in childhood.^{4,5} Herein, we present a case of GT in a young adult complicated with hemarthrosis after minor trauma, impacting mobility and physical activity, as reported by the patient. We present this case to highlight the rare association between hemarthrosis and GT and discuss the most convenient management plan for such cases.

Case Presentation

A 17-year-old male was diagnosed with Glanzmann thrombasthenia for one year, and the diagnosis was based on recurrent epistaxis and gingival hemorrhage. He experienced epistaxis episodes almost every 3–4 months, for which he received a platelet transfusion. He was assessed for other bleeding disorders; his von Willebrand's, factor VIII, and other clotting factor levels were all within normal limits, and his platelet count was within normal ranges. He had prolonged

bleeding time; the platelet function analyzer (PFA) showed prolonged closure time with both collagen and epinephrine at 211 s (normal up to 175 s) and collagen and adenosine diphosphate (ADP) at 233 s (normal up to 116 s). Platelets failed to aggregate with ADP, arachidonic acid, or collagen. The only response observed was with ristocetin (1.5 mg/mL).

In September 2018, the patient developed left ankle pain after twisting while playing sports; there was no direct trauma, swelling, or restricted joint movement. The patient visited a local health center. Physical examination was unremarkable without any effusion or other findings suggestive of hemarthrosis; labs results showed normal platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT); plain ankle radiography did not show any definite significant bone or articular pathology (Figure 1A); depending on these findings, there was no suspicion of an acute injury like hemarthrosis, and the patient was discharged with pain killers.

Four weeks later, the patient returned due to pain and ankle discomfort. Ankle MRI showed 2018 mild ankle joint effusion and synovitis, with focal mass-like synovial proliferation noted at the anterior ankle recess, showing mild enhancement and blooming artifacts (Figure 1A). The radiological findings were likely indicative of siderotic synovitis. After MRI was performed, the patient did not attend the follow-up appointments.

In December 2021, he visited the emergency department because of left ankle pain after the injury. The pain occurs almost monthly for one or two days, and then resolves with rest and analgesia. Examination revealed no swelling or gross deformity, but some tenderness was observed. Magnetic resonance imaging (MRI) showed remarkable subarticular cystic changes in the tibial and talar aspects of the ankle joint, with overlying articular cartilage changes and synovial thickening mainly anteriorly.

The patient was referred to an orthopedic clinic for review in February 2022. He reported that pain is usually bearable but increases with cold weather. No apparent swelling or tenderness was observed in the anterior aspect of the ankle. The images (Figure 1B) again demonstrated the same subarticular cystic-like changes with no interval changes. Owing to synovial changes, the patient was referred to a specialized ortho-oncology center for assessment in July 2022. Upon examination, the patient had a full range of motion, but experienced pain episodically. Magnetic resonance imaging

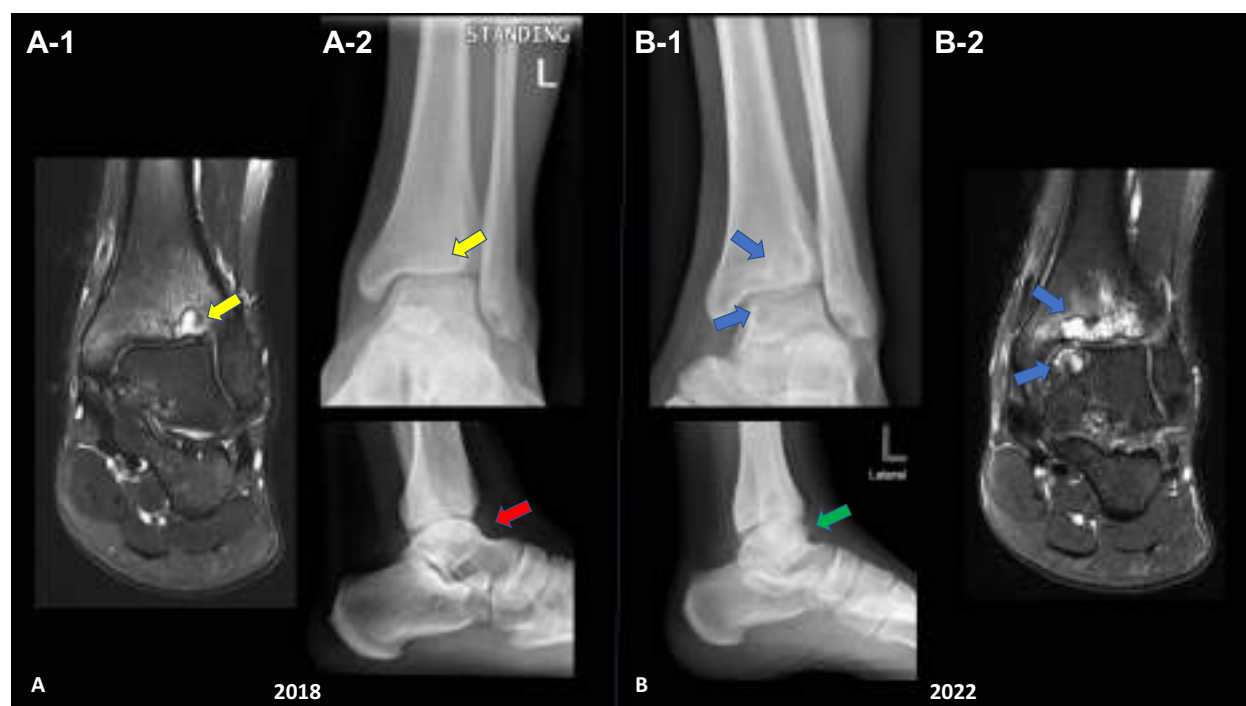


Figure 1 Comparative images: (A) Two sets of ankle radiographs (MRI A-1, Plain XR A-2) AP and lateral views first in 2018 showed mild ankle joint effusion (red arrow) and small tibial plafond subchondral cystic changes (yellow arrow). (B) 2022 follow-up radiographs (Plain XR B-1, MRI B-2) showed progressive changes with multiple large subchondral cysts involving the ankle joint opposing articular surfaces (blue arrows) as well as secondary osteoarthritis with osteophyte formation (green arrow) and reduced ankle joint space. Ankle MRI-selected images coronal PD FS are illustrating the progression of the articular pathology, increase the number of subchondral cysts.

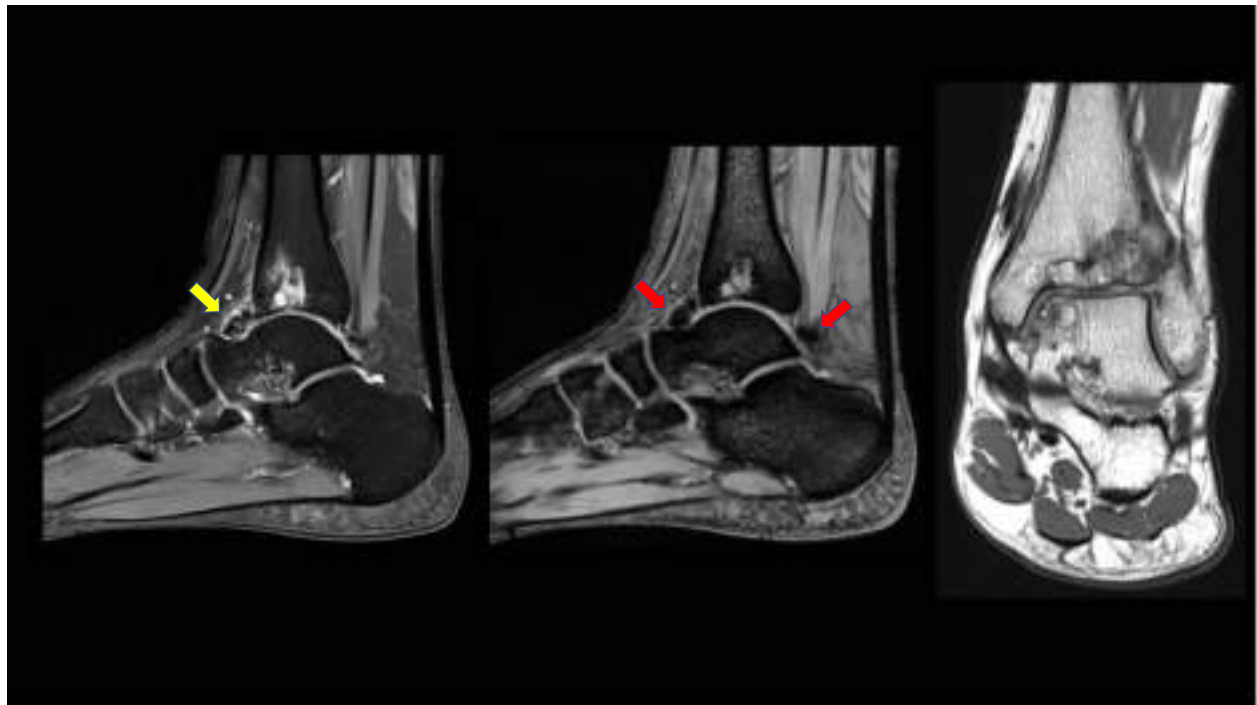


Figure 2 MRI of the ankle joint: sagittal T2WI, sagittal gradient sequence and coronal PDWI, show intra-articular synovial low signal intensity (yellow arrow) demonstrating blooming artifact on the gradient sequence (red arrows) in the anterior and posterior recess of the ankle joint consistent with blood degradation products related to repeated episodes of hemarthrosis. Again, secondary osteoarthritic changes are noted with multiple cystic changes/geodes with surrounding marrow oedema. Findings are consistent with siderotic chronic synovitis.

(MRI) was performed during the visit. MRI findings were consistent with siderotic synovitis in the anterior and posterior ankle joint recesses, with a mild reduction in interval size anteriorly. Tibiotalar hemophilic arthropathy with opposing subarticular pseudocyst formation and chondromalacia was observed (Figure 2).

Due to the recurrent presentations of pain, the orthopedic team performed an intraarticular steroid injection in December 2022. Arthrocentesis did not yield any blood, and the procedure was uneventful. On follow-up in January 2023, the patient reported reasonable pain control and no joint swelling and was advised to continue a tailored physical therapy regimen.

Discussion

Dr. Edward Glanzmann first described Glanzmann thrombasthenia in 1918, which is caused by a deficiency or abnormality of platelet glycoprotein IIB/IIIa, a surface protein that binds to either side of the fibrinogen molecule, allowing for platelet aggregation and the formation of a platelet plug at the site of the injury.³ In GT, this step does not occur properly; although the platelet count is generally normal, platelets cannot bind to fibrinogen and thus form a stable clot. The recognition of GT pathogenesis has improved our understanding of the role of platelets in homeostasis and their treatment and implantation.^{1,3}

GT typically presents in infancy or early childhood. The diagnosis is suspected after bouts of bleeding, mainly after trauma, and the bleeding episodes are disproportionate to the bleeding occurring in a normal patient. When GT is suspected, other common bleeding disorders must be excluded.^{3,4} Initial laboratory assessment showed normal to near-normal platelet count and morphology, normal prothrombin time, activated partial thromboplastin time, prolonged bleeding time, and abnormal measurements of advanced platelet function tests, such as.⁶ Family history is essential, as GT's incidence rate of GT is higher in certain ethnic groups due to consanguinity.⁷

Generally, bleeding due to platelet defects manifests as superficial bleeding symptoms and signs.⁸ In GT purpura and epistaxis are the most commonly encountered bleeds.^{6,8} Women are at high risk for menorrhagia and postpartum

bleeding.^{8,9} The involvement of other organs has also been reported.⁷ In one study, hemarthrosis due to GT was quite rare; 3% of patients with GT had hemarthrosis,^{5,10} bearing in mind the low incidence of GT itself. Joint involvement is significant because it affects the patient's functional status and quality of life.¹¹

Based on the existing literature, hemarthrosis and arthropathy pathophysiology of GT do not differ from hemophilic arthropathy.^{10,11} Although both are bleeding disorders, their patho-physiologies are not similar. Siderotic arthropathy is a condition characterized by iron deposition in the synovium and joint tissues, leading to joint damage and dysfunction. It is commonly associated with chronic hemarthrosis, which involves recurrent bleeding in the joint space.^{12,13} The pathophysiological process of hemarthrosis involves the participation of inflammatory cells, interleukins, neo-angiogenesis, and the formation of a protein complex called inflammasome, which contributes to the inflammatory response and subsequent damage to the synovial membrane.¹³ This, in turn, induces synovial hypertrophy and fibrosis and, in the long run, loss of cartilage and development of subchondral cysts.¹⁰

There is no cure for GT, and the mainstay of management is platelet transfusion and anti-fibrinolytic agents if bleeding episodes occur or in preparation for an invasive procedure.^{3,4} However, hemarthrosis management depends on its presentation and severity. In acute cases with swelling and limited range of motion, aspiration of the synovial space to relieve effusion is used alongside joint rest and splinting if needed,^{5,11} whereas in long-standing insults such as in our case, physical therapy and rehabilitation regimens have shown some benefit in preserving and restoring functional activity,^{11,14} with care to avoid trauma and medications that could further impair platelet function such as NSAIDs and aspirin.⁴ A surgical approach may be needed with severe joint destruction and failure of other conservative measures.¹¹ Our case is a rare entity that requires further studies through case reports, series, and reviews for a better understanding of the pathogenesis and course of the disease.

Conclusion

GT is a rare bleeding disorder stemming from GPIIb/IIIa platelet dysfunction that typically presents with mucocutaneous bleeding. However, the unusual association between hemarthrosis and chronic arthropathy, as exemplified in this case, highlights the potential complications that can arise in this condition. Recurrent ankle pain, diagnosed as siderotic synovitis and tibiotalar hemophilic arthropathy, underscores the significance of early recognition and tailored management strategies to address these rare but impactful manifestations. As our understanding of Glanzmann thrombasthenia continues to expand, it is crucial to conduct further research, including case reports and studies, to deepen our insight into its pathogenesis and clinical course, ultimately enhancing the care and quality of life of individuals with this condition.

Data Sharing Statement

Data and materials are available on request.

Statement of Ethics

Institutional approval obtained from Hamad Medical Corporation Medical Research Center, MRC-04-23-266.

Consent to Publish Statement

Written informed consent was obtained from the patient and his legal gradient (father) for the publication of this case report and any accompanying images.

Acknowledgments

The authors acknowledge the internal medicine residency program for its motivation and support.

Funding

This study was not supported by any funding. An open-access fee was provided by the Qatar National Library.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Lane DA, Philippou H, Huntington JA. Directing thrombin. *Blood*. 2005;106(8):2605–2612. doi:10.1182/blood-2005-04-1710
2. Chen JY, Chang KC, Lin YC, Chou HT, Hung JS. Safety and outcomes of short-term multiple femoral venous sheath placement in cardiac electrophysiological study and radiofrequency catheter ablation. *Jpn Heart J*. 2004;45(2):257–264. doi:10.1536/jhj.45.257
3. Solh T, Botsford A, Solh M. Glanzmann's thrombasthenia: pathogenesis, diagnosis, and current and emerging treatment options. *J Blood Med*. 2015;8:219–227. doi:10.2147/JBM.S71319
4. Sebastiano C, Bromberg M, Breen K, Hurford MT. Glanzmann's thrombasthenia: report of a case and review of the literature. *Int J Clin Exp Pathol*. 2010;3(4):443.
5. Klokorn RW, Lightsey AL Jr. Hemarthrosis associated with Glanzmann's thrombasthenia. *Arthritis Rheum*. 1979;22(12):1390–1393. doi:10.1002/art.1780221212
6. Botero JP, Lee K, Branchford BR, et al. ClinGen Platelet Disorder Variant Curation Expert Panel. *Haematologica*. 2020;105(4):888–894. doi:10.3324/haematol.2018.214239
7. Toogeh G, Sharifian R, Lak M, Safaei R, Artoni A, Peyvandi F. Presentation and pattern of symptoms in 382 patients with Glanzmann thrombasthenia in Iran. *Am J Hematol*. 2004;77(2):198–199. doi:10.1002/ajh.20159
8. George JN, Caen JP, Nurden AT. Glanzmann's thrombasthenia: the spectrum of clinical disease. *Blood*. 1990;75(7):1383–1395. doi:10.1182/blood.V75.7.1383.1383
9. Sherer DM, Lerner R. Glanzmann's thrombasthenia in pregnancy: a case and review of the literature. *Am J Perinatol*. 1999;16(06):297–301. doi:10.1055/s-2007-993875
10. Pontara E, Gresele P, Cattini MG, et al. Spontaneous hemarthrosis in combined Glanzmann thrombasthenia and type 2N von Willebrand disease. *Blood Coagulat Fibrinol*. 2014;25(4):401–404. doi:10.1097/MBC.0000000000000067
11. Lobet S, Hermans C, Lambert C. Optimal management of hemophilic arthropathy and hematomas. *J Blood Med*. 2014;17:207–218. doi:10.2147/JBM.S50644
12. Roosendaal G, Vianen ME, Marx JJ, Van Den Berg HM, Lefeber FP, Bijlsma JW. Blood-induced joint damage: a human in vitro study. *Arthritis Rheum*. 1999;42(5):1025–1032. doi:10.1002/1529-0131(199905)42:5<1025::AID-ANR23>3.0.CO;2-3
13. Costa F, Cordeiro ME, da Silva RL, et al. "Therapeutic options for hemarthrosis in severe hemophilic patients". *Medicina*. 2022;55:4.
14. Negrier C, Seuser A, Forsyth A, et al. The benefits of exercise for patients with haemophilia and recommendations for safe and effective physical activity. *Haemophilia*. 2013;19(4):487–498. doi:10.1111/hae.12118

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Acquired Clotting Factor Deficits During Treatment with Asparaginase in an Institutional Cohort

Vasiliki Papadopoulou & Giulia Schiavini

To cite this article: Vasiliki Papadopoulou & Giulia Schiavini (2023) Acquired Clotting Factor Deficits During Treatment with Asparaginase in an Institutional Cohort, Journal of Blood Medicine, , 569-574, DOI: [10.2147/JBM.S428159](https://doi.org/10.2147/JBM.S428159)

To link to this article: <https://doi.org/10.2147/JBM.S428159>



© 2023 Papadopoulou and Schiavini.



Published online: 07 Nov 2023.



Submit your article to this journal



Article views: 119



View related articles



View Crossmark data

Acquired Clotting Factor Deficits During Treatment with Asparaginase in an Institutional Cohort

Vasiliki Papadopoulou , Giulia Schiavini

Service and Laboratory of Hematology, Department of Oncology, Lausanne University Hospital, Lausanne, Switzerland

Correspondence: Vasiliki Papadopoulou, Lausanne University Hospital, Rue du Bugnon 46, Lausanne, 1011, Switzerland, Tel +41795560259, Email vasiliki.papadopoulou@chuv.ch

Abstract: We invariably see prolongation of activated partial thromboplastin time in patients treated with asparaginase in our clinical practice, but have noted that, contrary to hypofibrinogenemia and low antithrombin, clotting times' prolongation by asparaginase is largely unreported in the literature and guidelines and is not widely known to clinicians. We report on aPTT prolongations in a small cohort of patients, and on their origin, as investigated by measurements of clotting factors, fibrinogen, and D-dimers before and after asparaginase administration. We observed significant reductions in FIX and FXI (median post-treatment values of 27 IU/dl and 52 IU/dl, respectively), confirming one previous observation. A decrease in FXII was less pronounced but contributed to the prolonged aPTTs (FXII has no effect on in vivo haemostasis). The factor deficits are not due to consumption, as evidenced by unchanged D-dimer levels, and are, therefore, probably caused by disturbed factor synthesis. Our observations and insights contribute to elucidation of the profile of clotting assays during asparaginase treatment, and thus, to optimally monitor for undesirable events or steer situations of therapeutic anticoagulation without the risk of suboptimal or excessive anticoagulation.

Keywords: asparaginase, clotting assays, acute lymphoblastic leukemia, NK-cell lymphoma, bleeding, thrombosis

Introduction

Asparaginase, in various formulations (L-asparaginase, Pegaspargase, Erwinase), is a backbone drug for the treatment of acute lymphoblastic leukaemia (ALL) and is also used in regimens for aggressive NK-cell leukemia/lymphoma. It has specific toxicities, including hypofibrinogenemia, low antithrombin levels, and a thrombotic tendency, with a possibly high proportion of CNS thrombosis.¹ Hypofibrinogenemia and low antithrombin levels trigger administration of plasma/antithrombin products for severe deficits, according to different institutional practices, without solid evidence for cut-offs under which these products should be used.²

In our clinical practice, we invariably observe substantial prolongation of activated partial thromboplastin time (aPTT) in our patients with asparaginase, even in cases with low-normal fibrinogen levels, whose values alone do not justify aPTT prolongation. Moreover, in cases of concomitant heparin therapy, we usually observe excessive aPTT prolongations, with discordant aPTT and anti-Xa values. Prothrombin time (PT) prolongations are also present but are less pronounced. Contrary to hypofibrinogenemia and low antithrombin levels, prolongation of aPTT, or clotting times in general, by asparaginase, is not referenced in guidelines or clinical protocols, and we find it to be less common knowledge among clinicians. We were therefore interested to highlight this finding and to define the origin of aPTT prolongation and whether it mirrors a higher bleeding risk. To summarize the current knowledge on this topic, prolongation of coagulation screens has been published in the past, but only in very few studies dating four decades ago.^{3–5} Moreover, regarding individual clotting factor deficits under asparaginase treatment, to the best of our knowledge, the only references are ancient and come from a very limited number of patients. They have shown deficits of FIX and FXI during treatment (median measured values 62 and 48 IU/dL respectively) in a report on sixteen patients; in the same report, factors V, VII, VIII, X, II and XII were measured normal in most patients, with median values of 101, 150, 188,

116, 86 and 93 IU/dL, respectively, post-asparaginase. Factors II, IX, and X were measured low at 55, 47, and 63 IU/dL, respectively, in a second report we could find.^{6,7}

Methods

To characterize the aPTT prolongations we observe under asparaginase, we measured, in our clinical practice, clotting factors and D-Dimers, before and during the “asparaginase-phase”, in some of our ALL and NK-cell lymphoma patients treated with asparaginase-containing protocols in our institution from 01.09.20 to 01.09.22 (11 patients were treated in total, including 9 ALL and 2 NK-cell lymphoma; intrinsic pathway factors were measured in 6/11 cases, while factors II, V, VII, X were measured in 2/11 cases).

Results

Ten out of 11 (10/11) patients showed aPTT prolongation; the results of screening and factor assays on the designated day after the start of the drug are shown in [Table 1A](#) and [B](#). Highest aPTT values during the cycle are also shown. Patient 10, the only patient who received intramuscular pegaspargase, did not show prolonged aPTT or low fibrinogen levels during treatment. Three patients were anticoagulated, but their absolute aPTT value and aPTT ratio compared to the pre-asparaginase sample were excessive for their plasma heparin concentration measured by anti-Xa. Prothrombin times were moderately prolonged ([Table 1B](#)). We observed significant reductions in factors IX and XI with asparaginase in 6/6 patients in which these factors were measured, with a median decrease of 71% (median post-treatment value 27 IU/dL) for FIX and median decrease of 55% for FXI (median post-treatment value 52 IU/dL) ([Table 1A](#)). There were decreases of FVIII, but not to subnormal levels, as well as of FXII, with a median FXII reduction of 25% in the five non-inflammatory patients measured (all intrinsic pathway factors were measured in six patients in total, but one of the six patients was in inflammatory state at the post-treatment measurement, a state in which FXII levels decrease due to contact pathway activation, while FVIII levels increase as acute-phase reactant). The “common pathway” factors II, V and X, and the extrinsic pathway factor VII, were measured only in 2/11 patients, with average decreases in factors II, V, and X post-asparaginase of 29%, 40% and 50% respectively; no decrease in FVII was seen in these two patients. D-dimers, measured in all eleven cases, were not elevated compared to the pre-asparaginase status. Bleeding episodes were not observed.

Discussion

Based on our observations, we consider the acquired substantial deficits in FIX and FXI to be the main origin of the observed aPTT prolongations; these findings are in line with the two ancient reports cited in the introduction.^{6,7} Certainly, an isolated FIX at approximately 30 IU/dL, or, much less, an isolated FXI at approximately 50 IU/dL, would not be sufficient to provoke the aPTT prolongations observed, but the combined effect of multiple factor deficits, as well as of moderately low (low normal) fibrinogen levels, may produce visible aPTT prolongations. Moderately decreased levels of FXII also contribute to aPTT prolongation, given the sensitivity of the assay to this factor, which, however, does not play a role in *in vivo* hemostasis. Different asparaginase doses/schedules or formulations, as well as variable individual patient susceptibility, produce different coagulation factor nadirs, and the lowest levels are expected after consecutive doses. We observed no abnormal coagulation screens in the only patient who received pegaspargase intramuscularly, but we cannot conclude through only one case whether intramuscular administration attenuates the effects of the drug on coagulation. As for the moderate prolongation of prothrombin times, we suggest that they are brought about by moderate decreases in factors II, V, and X along with the moderately low fibrinogen, considering that we measured these factors in only two patients.

The mechanism of clotting factor depletion by asparaginase is unclear, as are the mechanisms of fibrinogen and antithrombin reduction, but research has shown that hypofibrinogenemia is not caused by peripheral consumption,⁸ and therefore a deficit of synthesis has to be assumed, and the same could be applied to the synthesis of multiple clotting factors. The non-elevation of D-dimer levels ([Table 1B](#)) after treatment in our patients excluded factor consumption, supporting this hypothesis. Indeed, asparaginase is known to alter hepatic metabolism, with the development of important hypertriglyceridemia, liver steatosis, and possible inhibition of protein synthesis, with unclear mechanisms probably related to depletion of glutamine and asparagine.⁹ The mechanism by which FIX seems to be more affected than other factors in our small cohort is unclear; however, the degree of acquired deficits should be precisely quantified with

Table 1 Coagulation Parameters Before Start and After Administration of Asparaginase in the Patient Cohort

(A)																		
Patients/Diagnosis		aPTT (sec)			FVIII (IU/dl)			FIX (IU/dl)			FXI (IU/dl)			FXII (IU/dl)			Fibrinogen (g/l)	
		Pre	Post	AntiXa IU/ mL	Pre	Post	D(FVIII)	Pre	Post	D(FIX)	Pre	Post	D(FXI)	Pre	Post	D(FXII)	Pre	Post
Pt 1	T-ALL (26, m)	29	49 (d7) Max. 54 (d8)	0	176	113	−36%	90	26	−71%	105	56	−47%	85	66	−22%	1.8	1.3
Pt 2 ♣	B-ALL (20, f)	36	101 (d5) (Max.)	0.4	130	130	0	70	19	−73%	120	49	−59%	125	65	−48%	2.4	1.6
Pt 3 ♣	T-ALL (38, f)	29	67 (d3) Max. 70 (d6, antiXa 0.5)	0.5	229	168	−27%	120	57	−53%	130	75	−42%	140	105	−25%	2.1	1.4
Pt 4	T-ALL (55, m)	30	42 (d6) (Max.)	0	91	75	−18%	95	27	−72%	110	55	−50%	85	69	−19%	3.8	1.6
Pt 5	B-ALL (19, m)	27	51 (d10) (Max.)	0	175	125	−29%	80	23	−71%	95	25	−74%	75	53	−29%	2.8	1.0
Pt 6 ♣	B-ALL (20, f)	24	89 (d10) (Max.)	0.15	119	155	+30%	115	36	−69%	105	37	−65%	175	37	−79%	1.5	4.1
Pt 7	B-ALL (64, f)	29	49 (d8) (Max.)	0	ND	ND		ND	ND		ND	ND		ND	ND		5.2	2.4
Pt 8	T-ALL (36, m)	31	77 (d22) (Max.)	0	ND	ND		ND	ND		ND	ND		ND	ND		1.8	1.5
Pt 9	NK (63, f)	35	127 (d12) (Max.)	0	ND	ND		ND	ND		ND	ND		ND	ND		5.5	1.4
Pt 10	NK (62, m)	27	37 (d21) (Max.)	0	ND	ND		ND	ND		ND	ND		ND	ND		2.1	1.8
Pt 11	T-ALL (36, m)	28	48 (d10) (Max.)	0	ND	ND		ND	ND		ND	ND		ND	ND		1.3	1.5

(Continued)

Table 1 (Continued).

(B)																			
	INR (PT sec)		Antithrombin (%)			FII (IU/dl)			FV (IU/dl)			FVII (IU/dl)			FX (IU/dl)			D-Dimers (ng/mL)	
	Pre	Post	Pre	Post	D(AT)	Pre	Post	D (FII)	Pre	Post	D(FV)	Pre	Post	D(FVII)	Pre	Post	D(FX)	Pre	Post
Pt 1	1.1 (11)	1.2 (d7) (12.5)	91	53	−42%	90	69	−23%	130	95	−27%	75	95	+27%	80	50	−38%	1440	1494
Pt 2❖	1.1 (11.6)	1.2 (d5) (13.1)	62	33	−47%	ND	ND		ND	ND		ND	ND		ND	ND		394	288
Pt 3❖	1.0 (10.5)	1.0 (d3) (11.4)	86	57	−34%	ND	ND		ND	ND		ND	ND		ND	ND		1023	1023
Pt 4	1.1 (11.9)	1.2 (d6) (13.4)	92	62	−32%	ND	ND		ND	ND		ND	ND		ND	ND		1651	938
Pt 5	1.1 (12.1)	1.5 (d10) (15.5)	81	53	−35%	63	41	−35%	100	47	−53%	48	51	+6%	80	31	−61%	1621	211
Pt 6❖* 	1.0 (10.8)	1.1 (d10) (12.2)	98	51	−48%	ND	ND		ND	ND		ND	ND		ND	ND		3128	1477
Pt 7	1.0 (10.6)	1.1 (d8) (11.9)	82	56	−32%	ND	ND		ND	ND		ND	ND		ND	ND		3128	1477
Pt 8	1.0 (11)	1.2 (d22) (12.4)	107	61	−43%	ND	ND		ND	ND		ND	ND		ND	ND		1941	2180
Pt 9	1.0 (10.6)	1.3 (d12) (14)	102	41	−60%	ND	ND		ND	ND		ND	ND		ND	ND		1977	1249
Pt 10	1.0 (11.0)	1.1 (d21) (12.1)	ND	53	ND	ND	ND		ND	ND		ND	ND		ND	ND		4376	1378
Pt 11	1.1 (11.4)	1.1 (d10) (12.1)	88	41	−53%	ND	ND		ND	ND		ND	ND		ND	ND		277	312

Notes: Pre-asparaginase samples: taken hours before first administration of the drug. Post-asparaginase samples: taken on designated day after start of the drug. Drug formulation was: L-asparaginase 6000 IU/m² iv on days 1, 3, 5, 13, 15, 17, 19, and 21 for patients 1, 2, 3, 4, 8 and 11 (asparaginase effects persist for 72h and may be additive with repetitive doses) / L-asparaginase 6000 IU/m² iv on days 1, 3, 5, 7, 9, 11, and 13 for patient 9 / Pegaspargase iv 2000 IU/m² on d1 for patients 5 and 6 (half-life varying from 6 days –2 weeks) / Pegaspargase iv 500 IU/m² on d1 for patient 7 (low dose, due to age) / Pegaspargase 2500 IU/m² im on d1 and d13 for patient 10. Day 1 is 1st day of asparaginase administration for all patients, not the 1st day of the multi-agent regimen. The regimen for ALL patients (induction or late intensification) contained daunorubicin, vincristine, prednisone, cyclophosphamide, and asparaginase, according to GRAALL-2005.¹⁶ For NK-cell lymphoma cases: SMILE regimen for patient 9¹⁷ and pegaspargase-gemcitabine-oxaliplatin for patient 10. All patients had normal liver function except for grade I transaminitis in patient 9. * Denotes patients therapeutically anticoagulated by unfractionated heparin at the timepoint of the post-treatment sample (1 PE and 2 DVT; all occurring before start of asparaginase). Factor measurement results were independent of the presence of heparin. All pre-treatment samples, as well as the post-treatment samples of all other patients, were drawn under prophylactic perfusion of 100 iu/kg heparin for/24h, with undetectable anti-Xa. * Patient 6 was in systemic inflammatory state (febrile bacteremia, without disseminated intravascular coagulation) at collection of the post-treatment sample. **A.** aPTT prolongation was observed in all patients except patient 10, with excessive prolongation in anticoagulated patients. Anti-Xa is shown for therapeutically anticoagulated patients post-asparaginase. Significant decreases in FIX and FXI are observed, with median reductions of 71% for FXI and 55% for FXI. FVIII is upregulated and FXII is downregulated in inflammatory states; therefore, they are not reliable to assess the effect of asparaginase in patient 6. These two factors did not show deep decreases post-asparaginase in the five non-inflammatory patients, with median decreases of 27% and 25%, respectively. Fibrinogen levels were invariably lower after treatment, except for patient 6, for whom it was elevated as an acute-phase reactant, and for patient 10, who showed no prolongation of coagulation screens. Highest aPTT values during the cycle are also shown (as Max.). **B.** PTs were moderately prolonged. There were no significant changes in D-dimers' levels. Important decreases in antithrombin observed in all patients. Decreases in FII, FV, and FX, but not in FVII, were observed in two patients.

prospective data in larger samples, with measurements at defined time points and constant drug doses. FVIII, which seems less affected than other factors in our cohort, is synthesized mostly by endothelial cells; therefore, it is unknown whether the effect of asparaginase applies to its synthesis. As for FVII, which seems unaffected, we cannot generalize the findings as we measured it only in two cases.

Data on the bleeding risk with asparaginase in adults are limited, whereas a thrombotic risk related to asparaginase is rather widely reported;¹⁰ therefore, it is uncertain whether the observed factor deficits could provoke a bleeding tendency during treatment. In the large prospective MRC UKALL XII trial, an extremely low number of treatment-related deaths were reported in general, while the GRAALL 2005 prospective trial showed an incidence of grade 3–4 bleeding at 4% during induction.^{11,12} In large prospective trials in children, bleeding-related deaths were rare.¹³ A recent retrospective study on pegaspargase toxicities in 57 adults, published in abstract form, showed a frequency of all-grade bleeding episodes during induction at 7% and during post-induction at 10%, without available correlative data for fibrinogen levels or clotting times during induction.¹⁴ In another retrospective study, the incidence of any-grade bleeding was 5.3%, with no CNS bleeding, and was higher during induction, possibly due to concomitant thrombocytopenia.¹⁵ It is important to remember that not only clotting factors but also natural anticoagulants (protein C, protein S, antithrombin) are decreased by asparaginase,^{1,7} which can reduce the risk of bleeding. Thrombin generation potential was not decreased in asparaginase-treated patients in a relevant study, and interestingly, it was not substantially influenced by fibrinogen or antithrombin levels.¹⁶

Conclusions

In summary, we report aPTT prolongations with asparaginase, secondary to acquired deficits of multiple clotting factors, due to globally impaired clotting factor synthesis, with factors IX and XI seemingly affected to a greater extent than others. A limitation of our results, except for the small number of cases in our series, is that factor measurements were not performed at highest aPTT/PT value of the cycle for all cases, so nadir factor values could be lower than those measured in some cases. Our observations confirm long-dating underemphasized data^{5–7} and are relevant for the understanding of the laboratory profile of patients treated with asparaginase, and therefore for their optimal assessment. Moreover, they suggest that, if aPTT is used to monitor therapeutic heparin in patients during the asparaginase phase of treatment, the aPTT ratio should be interpreted carefully; a denominator value taken pre-asparaginase can provide an excessive ratio and give impression of excessive anticoagulation. However, one could indeed ask whether a bleeding risk is present when very high absolute aPTT values (a result of the deficits of clotting factors and fibrinogen, in combination with anticoagulation) are found in this context. The levels of factors measured in our patients are not a contraindication to full-dose anticoagulation when the latter is indicated, and general experience with bleeding risk with asparaginase is benign; however, in cases of clotting assays implying severe factor depletion (which could result after consecutive asparaginase doses or in very susceptible patients), close clinical and laboratory monitoring for bleeding risk under anticoagulation is advisable, even more so in the simultaneous presence of thrombopenia due to myelosuppression.

Data Sharing Statement

More detailed clinical/laboratory data are available upon request.

Ethics Statement

Report of this case series was approved by the cantonal Ethics Committee of the Canton of Vaud, Switzerland (CER-VD, No 2022-02114). All patients had given consent for anonymous reutilization of data.

Acknowledgments

The authors wish to thank Mr. Francisco-Xavier Gomez, lead laboratory technician at the Hemostasis Laboratory of Lausanne University Hospital.

Author Contributions

Both authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

No external funding was relevant to this publication.

Disclosure

The authors have no conflicts of interest to declare.

References

1. Truelove E, Fielding AK, Hunt BJ. The coagulopathy and thrombotic risk associated with L-asparaginase treatment in adults with acute lymphoblastic leukaemia. *Leukemia*. 2013;27(3):553–559. doi:10.1038/leu.2012.290
2. Zwicker JJ, Wang TF, DeAngelo DJ, et al. The prevention and management of asparaginase-related venous thromboembolism in adults: guidance from the SSC on Hemostasis and Malignancy of the ISTH. *J Thromb Haemost*. 2020;18(2):278–284. doi:10.1111/jth.14671
3. Pui CH, Jackson CW, Chesney C, et al. Sequential changes in platelet function and coagulation in leukemic children treated with L-asparaginase, prednisone, and vincristine. *J Clin Oncol*. 1983;1(6):380–385. doi:10.1200/JCO.1983.1.6.380
4. Homans AC, Rybak ME, Baglini RL, Tiarks C, Steiner ME, Forman EN. Effect of L-asparaginase administration on coagulation and platelet function in children with leukemia. *J Clin Oncol*. 1987;5(5):811–817. doi:10.1200/JCO.1987.5.5.811
5. Priest JR, Ramsay NK, Bennett AJ, Krivit W, Edson JR. The effect of L-asparaginase on antithrombin, plasminogen, and plasma coagulation during therapy for acute lymphoblastic leukemia. *J Pediatr*. 1982;100(6):990–995. doi:10.1016/S0022-3476(82)80536-2
6. Ramsay NK, Coccia PF, Krivit W, Nesbit ME, Edson JR. The effect of L-asparaginase of plasma coagulation factors in acute lymphoblastic leukemia. *Cancer*. 1977;40(4):1398–1401.
7. Bezeaud A, Drouet L, Leverger G, Griffin JH, Guillin MC. Effect of L-asparaginase therapy for acute lymphoblastic leukemia on plasma vitamin K-dependent coagulation factors and inhibitors. *J Pediatr*. 1986;108(5 Pt 1):698–701. doi:10.1016/S0022-3476(86)81044-7
8. Bettigole RE, Himmelstein ES, Oettgen HF, Clifford GO. Hypofibrinogenemia due to L-asparaginase: studies of fibrinogen survival using autologous 131-I-fibrinogen. *Blood*. 1970;35(2):195–200. doi:10.1182/blood.V35.2.195.195
9. Ollenschläger G, Roth E, Linkesch W, Jansen S, Simmel A, Modder B. Asparaginase-induced derangements of glutamine metabolism: the pathogenetic basis for some drug-related side-effects. *Eur J Clin Invest*. 1988;18(5):512–516. doi:10.1111/j.1365-2362.1988.tb01049.x
10. Goyal G, Bhatt VR. L-asparaginase and venous thromboembolism in acute lymphocytic leukemia. *Fut Oncol*. 2015;11(17):2459–2470. doi:10.2217/fon.15.114
11. Rowe JM, Buck G, Burnett AK, et al. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood*. 2005;106(12):3760–3767. doi:10.1182/blood-2005-04-1623
12. Orvain C, Balsat M, Tavernier E, et al. Thromboembolism prophylaxis in adult patients with acute lymphoblastic leukemia treated in the GRAALL-2005 study. *Blood*. 2020;136(3):328–338. doi:10.1182/blood.2020004919
13. Wheeler K, Chessells JM, Bailey CC, Richards SM. Treatment related deaths during induction and in first remission in acute lymphoblastic leukaemia: MRC UKALL X. *Arch Dis Child*. 1996;74(2):101–107. doi:10.1136/adc.74.2.101
14. Ben Mautner EP, Mort J, DiBenedetto S, et al. The risks of thrombotic and bleeding events in patients receiving PEGylated asparaginase for treatment of acute lymphoblastic leukemia. *J Clin Oncol*. 2022;40(16):e19015.
15. Aldoss I, Douer D, Behrendt CE, et al. Toxicity profile of repeated doses of PEG-asparaginase incorporated into a pediatric-type regimen for adult acute lymphoblastic leukemia. *Eur J Haematol*. 2016;96(4):375–380. doi:10.1111/ejh.12600
16. Lejhancova-Tousovska K, Zapletal O, Vytiskova S, Strbackova P, Sterba J. Profile of thrombin generation in children with acute lymphoblastic leukemia treated by Berlin-Frankfurt-Munster (BFM) protocols. *Blood Coagul Fibrinolysis*. 2012;23(2):144–154. doi:10.1097/MBC.0b013e32834fb539
17. Yamaguchi M et al. (2011). Phase II study of SMILE chemotherapy for newly diagnosed stage IV, relapsed, or refractory extranodal natural killer (NK)/T-cell lymphoma, nasal type: the NK-Cell tumor study group study. *J Clin Oncol*, 29(33), 4410–6. doi:10.1200/JCO.2011.35.6287

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Prevalence and Associated Factors of Anemia Among Hospital Admitted Patients in Eastern Ethiopia

Mohammed Umer Yusuf, Nuredin Abdurahman, Haftu Asmerom, Tesfaye Atsbaha, Adisu Alemu & Fitsum Weldegebreal

To cite this article: Mohammed Umer Yusuf, Nuredin Abdurahman, Haftu Asmerom, Tesfaye Atsbaha, Adisu Alemu & Fitsum Weldegebreal (2023) Prevalence and Associated Factors of Anemia Among Hospital Admitted Patients in Eastern Ethiopia, Journal of Blood Medicine, , 575-588, DOI: [10.2147/JBM.S431047](https://doi.org/10.2147/JBM.S431047)

To link to this article: <https://doi.org/10.2147/JBM.S431047>



© 2023 Yusuf et al.



Published online: 15 Nov 2023.



Submit your article to this journal



Article views: 609



View related articles



View Crossmark data

Prevalence and Associated Factors of Anemia Among Hospital Admitted Patients in Eastern Ethiopia

Mohammed Umer Yusuf¹, Nuredin Abdurahman¹, Haftu Asmerom², Tesfaye Atsbaha¹, Adisu Alemu³, Fitsum Weldegebreal²

¹Department of Internal Medicine, School of Medicine, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ²School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ³Department of Pathology, School of Medicine, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

Correspondence: Haftu Asmerom, School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, PO BOX 235, Harar, Ethiopia, Tel +251910089878, Email wawhaftu12@gmail.com

Background: Anemia is one of the most common comorbidities frequently seen in admitted patients. However, there is a scarcity of evidence regarding anemia among hospital admitted patients in Ethiopia, particularly in the Harari Region. Therefore, this study aimed to assess the prevalence and associated factors of anemia among hospital admitted patients in Eastern Ethiopia.

Methods: A hospital-based cross-sectional study was conducted from October 25 to December 30, 2022. Four milliliters of venous blood were collected and complete blood count was done using the DxH 800 (Beckman Coulter, Inc, Miami, FL) hematology analyzer. The data were entered in Epi-data version 4 and exported to SPSS version 26 for statistical analysis. Bivariable and multivariable logistic regression models were fitted. The level of significance was declared at a p-value of < 0.05.

Results: Of the 381 hospital admitted patients, 64.8% (95% CI = 60.01, 69.65) of the participants were anemic. Admitted patients who drank standard alcohol daily (AOR = 3.78, 95% CI = 1.71, 8.30), underweight (AOR = 9.39, 95% CI = 2.90, 30.46), and undernourished patients (AOR = 2.59, 95% CI = 1.15, 5.84), patients admitted with chronic kidney disease (AOR = 11.16, 95% CI = 4.06, 30.64), chronic liver disease (AOR = 3.20, 95% CI = 1.21, 8.47), deep vein thrombosis (AOR = 6.22, 95% CI = 1.98, 19.52), infectious disease (AOR = 9.71, 95% CI = 2.77, 34.02), and chronic non-communicable disease (AOR = 7.01, 95% CI = 1.90, 25.99) were all significantly associated with anemia.

Conclusion: Anemia was common among hospital admitted patients and should prompt the focus on admission diagnoses that are likely to play leading roles in etiology. This information indicates a need for routine screening of anemia for all admitted patients to improve their health.

Keywords: anemia, prevalence, associated factors, admitted patients, Ethiopia

Introduction

The majority of hospital admitted patients are adults and geriatrics (≥ 15 years old) with chronic diseases and several comorbidities, which cause frequent hospitalizations.¹ Anemia is one of the more prevalent comorbidities.^{2–6} It is a condition in which the number of red blood cells (RBC) or hemoglobin (Hgb) concentration within them is lower than normal.⁷ Accordingly, it is diagnosed in non-pregnant women ≥ 15 years old Hgb < 12.0 g/dl; men ≥ 15 years old Hgb < 13.0g/dl.⁸ Factors that contribute to the onset of anemia in admitted patients include nutritional deficiencies (iron, folate, and vitamin B12), acute or chronic blood loss, chronic diseases, hemoglobinopathies, infectious diseases (malaria, tuberculosis, the Human Immune Virus (HIV), and parasitic infections), and even other unknown causes.⁹

Compared to the general population, admitted patients have a higher prevalence of anemia.¹⁰ It is thought to affect ranging from 30% to 90% of newly admitted patients over the duration of their illnesses.^{2–6,11,12} Its prevalence is higher in female subjects, from 15 years of age to adulthood, and in older (> 80 years old) patients of both genders, affecting

42% of subjects.¹³ The most typical cause of anemia worldwide is iron deficiency anemia (IDA).¹⁴ However, multifactorial anemia includes anemia of chronic diseases (ACD), chronic kidney disease (CKD), dietary deficiencies, hemorrhages, use of antithrombotic medications, inflammation of iron metabolism,^{15,16} and anemia of chronic diseases (ACD), which have a higher prevalence of anemia.¹³ Recent research has shown that ACD and IDA, both of which have low iron stores, have altered iron metabolism.¹⁷ Hepcidin, a hormone produced by the liver during chronic inflammation, rises significantly when ferroportin activity is inhibited by hepcidin, which reduce iron absorption in the intestine while simultaneously causing iron sequestration in macrophages and enterocytes.¹⁸

Data from previous studies conducted in Ethiopia showed that the prevalence of anemia was higher in hospitalized patients.^{19,20} Despite the high prevalence of anemia in admitted patients, very little research has been conducted in our setup. Therefore, the purpose of this study was to determine the prevalence of anemia and its contributing factors among hospital admitted patients in Eastern Ethiopia.

Materials and Methods

Study Design, Area, and Period

A hospital-based prospective cross-sectional study was conducted in Harar Town, one of the Federal Democratic Republic of Ethiopia's regional states in the country's east. Harari Region is one of Ethiopia's nine regional states, with Harar as its capital city. It is located 526 kilometers east of Addis Ababa, Ethiopia's capital. There are four public hospitals, two private hospitals, and five health centers in the region. Among these hospitals, the study was conducted at two public hospitals in the region (Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH) and Jugol General Hospital) and one private hospital (Harar General Hospital). These hospitals provide curative, rehabilitative, and preventive health care services. The study was conducted at these three selected hospitals from October 25 to December 30, 2022.

Population, Inclusion, and Exclusion Criteria

The study population included all individuals over the age of 15 who were hospitalized at the selected hospitals and agreed to participate in the study. The admission diagnosis of the patients was obtained by reviewing the patient's card or chart. On the other hand, pregnant women and individuals who were unable to communicate were excluded from the study.

Sample Size Determination and Sampling Technique

The sample size of study participants was determined using a single population proportion formula by considering the 34% prevalence of anemia among hospital-admitted patients from a previous study conducted in Ethiopia.¹⁹

$$N = \frac{Z^2 P(1 - P)}{d^2}$$

where n represents the minimum sample size required; z is 1.96, which is the standard normal value at the level of confidence desired (for a 95% confidence interval); d is 0.05, the acceptable error willing to be committed; and p, which is 0.34, is the estimated proportion of anemia prevalence in hospital admitted patients.

Based on the above assumption, the sample size was calculated as follows:

$$\frac{1.96^2 0.34(1 - 0.34)}{0.05^2} = 345$$

After adding 10% non-respondent rates, a total of 381 study participants were recruited using a simple random sampling technique. Sampling frames were created and the number of patients to be included in the study was determined for each of the Hospitals by probability proportion allocation to size sampling, followed by simple random sampling (Figure 1).

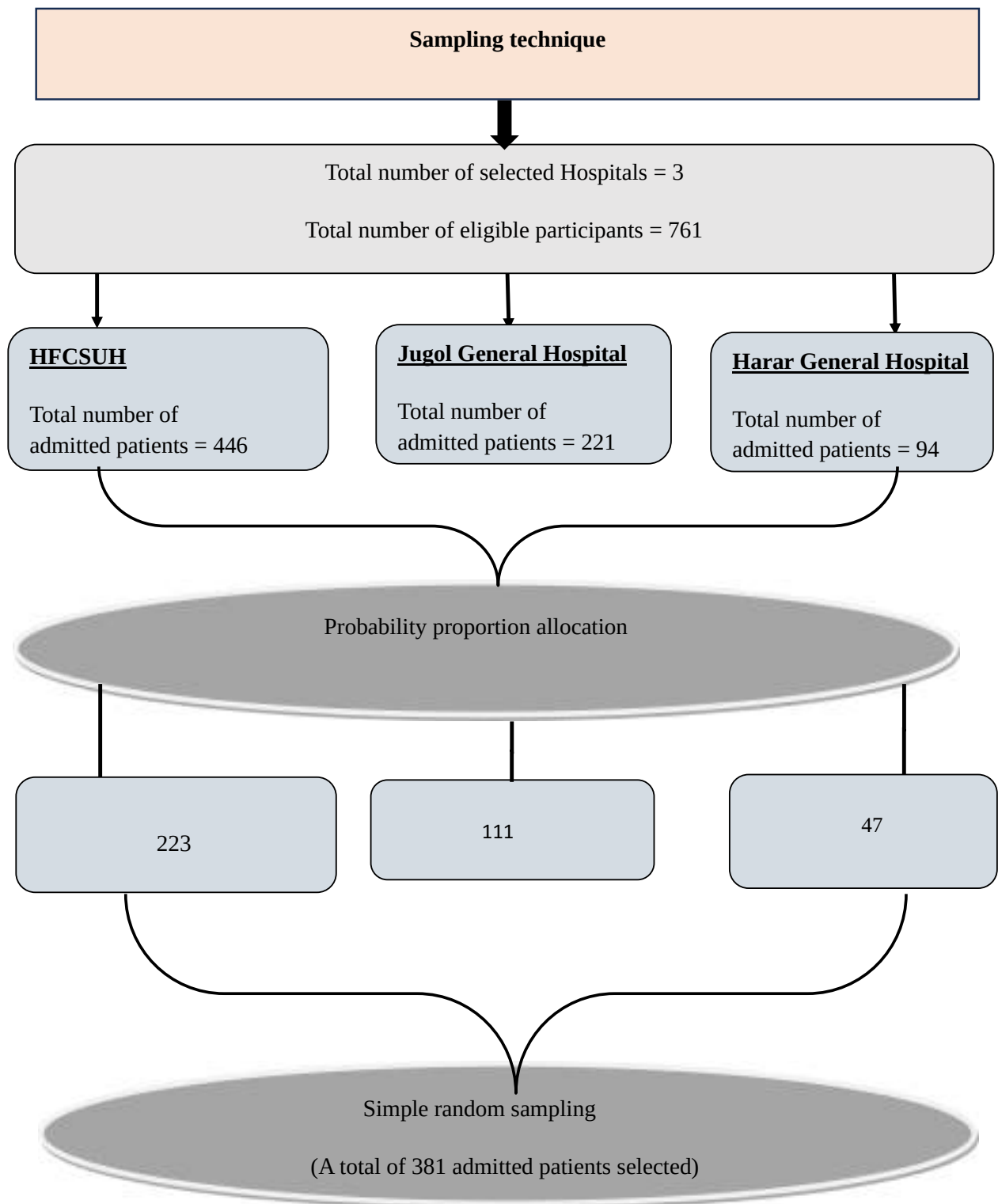


Figure 1 Schematic presentation of sampling procedure among hospital admitted patients in Eastern Ethiopia, 2022.

Data Collection and Measurement

A Structured questionnaire was prepared from different literature.^{3,4,11,21,22} A total of nine data collectors (one senior clinical nurse, one senior medical resident, and one senior medical laboratory professional) from each of the selected hospitals collected socio-demographic characteristics, lifestyle, and history and clinical conditions (age at admission, cause of hospital admission, and laboratory tests) of each patient's data. The study participants' weight and height were measured using a digital weight scale with a connected height scale (Adult Scale ASTOR). Every day, the scale was reset to zero and calibrated. The heights of the participants were measured to the closest 0.1 cm. The body mass index (BMI) of the subjects was calculated by dividing their weight (in kilograms) by their height (in meters squared), and they were classified as underweight (< 18.5 kg/m²), normal (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²), and obese (≥ 30 kg/m²).²³

The middle upper arm circumference (MUAC) was used to measure nutritional status. With no clothing on the arm, the left arm circumference was measured in triplicate using a non-stretchable standard MUAC tape to the nearest 0.1 cm. Three measurements were obtained and the average was calculated. A standard scale was used to quantify the MUAC at the midpoint between the acromion and the shoulder. The mean of the two nearest recorded measures was utilized when the variation surpassed the average values of the three measures. Patients admitted with MUACs of < 22 cm were considered undernourished, whereas MUACs of ≥ 22 cm were regarded as normal.²⁴ One standard alcohol drink was defined as a drink with a net alcohol content of approximately 10g of ethanol (for example, one standard bottle of regular beer (285 mL), one single measure of spirits (30 mL), and one medium-sized glass of wine (120 mL)). Cigarette smoking was defined as admitted patients who had smoked at least one stick of cigarettes per day in the month (30 days) preceding data collection. Khat chewing was defined as admitted patients who had chewed khat in the month (30 days) before data collection.

Sample Collection and Laboratory Analysis

Four mL of venous blood was drawn using venipuncture according to World Health Organization (WHO) protocol²⁵ under aseptic conditions from each admitted patient. The blood samples were then put into an ethylene diamine tetraacetic acid (EDTA) tube and gently mixed by inverting the tube several times to avoid clotting. An automated hematology analyzer, UniCel DxH 800 Coulter (Beckman Coulter, Inc, Miami, FL) was used for the determination of Hgb, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).

Diagnosis of Anemia

Anemia was defined according to WHO criteria,²⁶ with a Hgb value of < 13 g/dl for males and < 12 g/dl for females. Anemia severity was also classified as mild (males: 11–12.9 g/dl; females: 11–11.9 g/dl), moderate (Hgb between 7 and 10.9 g/dl in both males and females), and severe (Hgb levels ≤ 7 g/dl in both males and females). Anemia was also classified into different morphology classifications based on MCV and MCHC values: microcytic hypochromic anemia (MCV < 80 fl and MCHC $< 32\%$), macrocytic normochromic anemia (MCV > 100 fl and MCHC between 32% and 36%), and normocytic normochromic anemia (MCV between 80 fl and 100 fl and MCHC between 32% and 36%).²⁷

Data Processing and Analysis

Following data collection, the data were edited and cleaned, and each questionnaire was coded and validated for completeness. Epi-Data version 4.6 was used to enter data into the computer, and the statistical program Statistical Package for Social Sciences (SPSS) version 26 was used for analysis. Frequency, percentages, tables, and figures were used to describe categorical variables. The Kolmogorov-Smirnov test was used to ensure that continuous data had a normal distribution. Bivariable logistic regression analysis was employed, and the Crude Odds Ratio (COR) with 95% CI was computed to analyze the relationship between each independent variable and the outcome variable. Variables with p-values less than 0.25 were included for multivariable logistic regression analysis. Model goodness-of-fit was checked by the Hosmer and Lemeshow test, and the final model was well-fitted with the included independent variables (p-value = 0.81). The final model was performed to control the confounding variables and identify the associated factors by estimating the Adjusted Odds Ratio (AOR) with a 95% CI. Statistical significance was declared at a p-value of < 0.05 .

Data Quality Control

A questionnaire was created in English and then translated into the local languages (Afan Oromo and Amharic) and then returned to English to maintain its consistency. One week before the actual data collection, 5% of the questionnaires were pre-tested among patients admitted at Dilchora General Hospital, which is about 52 kilometers away from the study region. The investigators attentively monitored the data collectors. The investigators evaluated the completeness of each questionnaire daily.

Ethical Consideration

The study protocol was approved by the Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University's College of Health and Medical Sciences, with reference number (IHRERC-175-2022). We conducted the investigations in accordance with the Helsinki Declaration's principles. The head of each hospital and participant above the age of 18 years provided informed, voluntary, written, and signed consent. In addition, written informed consent was obtained from the child's parent/legal guardian and oral assent was obtained from children under 18 years of age after explaining the benefits and risks of the study. Personal identifiers were not included during the data collection to ensure anonymity.

Results

Socio-Demographic Characteristics

A total of 381 admitted patients took part in the study. One hundred ninety-eight (52.0%) participants were female. The ages of the study participants ranged from 15 to 90 years old, with a mean age of 40.98 (SD = 17.3). The majority of 210 (55.1%) lived in rural areas. Regarding educational status, 82 (21.5%) admitted patients were in secondary school, and 70 (18.4%) were in college or above (Table 1).

Nutritional and Behavioral-Related Factors

One hundred fourteen (29.9%) of the 381 admitted patients were underweight (BMI < 18.5 kg/m²). In contrast, 59 (15.5%) of the patients who were admitted were obese (BMI ≥ 30 kg/m²). One hundred seventy admitted patients (44.6%) had an undernourished condition (MUAC 22 cm). According to the study, 245 (64.3%) of the participants were cigarette smokers, with 125 (32.8%) of them smoking ≥ 5 cigarette sticks each day. Additionally, 223 (58.5%) of the

Table 1 Sociodemographic Characteristics of Hospital Admitted Patients in Eastern Ethiopia, 2022

Variables	Category	Admitted Patients n (%)
Age in years	15–24	67 (17.6)
	25–34	120 (31.5)
	35–44	55 (14.4)
	45–54	48 (12.6)
	55–64	63 (16.5)
	Above 65	28 (7.3)
Sex	Female	198 (52.0)
	Male	183 (48.0)
Residence	Urban	171 (44.9)
	Rural	210 (55.1)
Educational Status	Unable to read and write	75 (19.7)
	Able to read and write	80 (21.0)
	Preparatory School	74 (19.4)
	Secondary School	82 (21.5)
	College or above	70 (18.4)

Table 2 Nutritional and Behavioral Related Factors of Hospital Admitted Patients in Eastern Ethiopia, 2022

Variables	Categories	N (%)
Body mass index	Underweight	114 (29.9)
	Normal weight	114 (29.9)
	Overweight	94 (24.7)
	Obese	59 (15.5)
MUAC	Malnourished	170 (44.6)
	Normal	211 (55.4)
Amount of standard Alcohol drink	No	158 (41.5)
	1– 4 days per a week	90 (23.6)
	Daily	133 (34.9)
Khat chewing habit	No	173 (45.4)
	< 300 g per day	119 (31.2)
	≥ 300 g per day	89 (23.4)
Cigarette smoking	No	136 (35.7)
	< 5 cigarette sticks per day	120 (31.5)
	≥ 5 cigarette sticks per day	125 (32.8)

Abbreviation: MUAC, Middle Upper Arm Circumference.

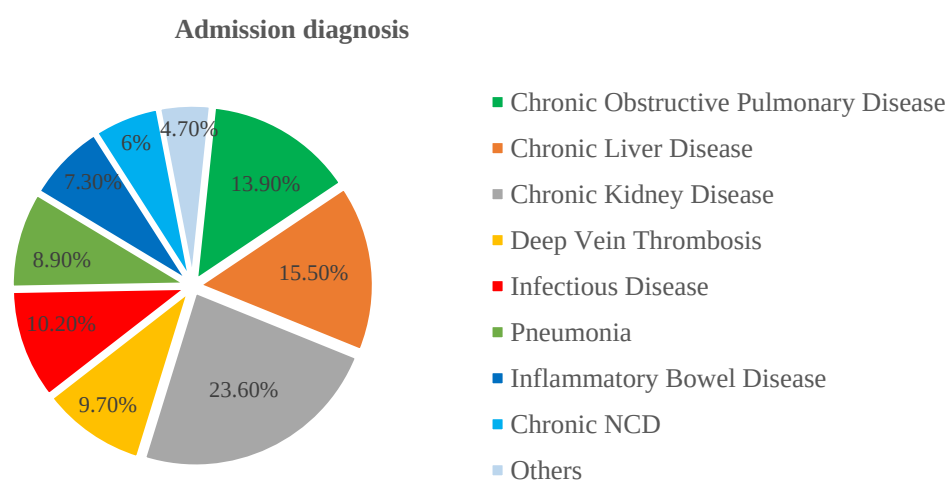
study participants were current alcohol users, with 133 (34.9%) of them consuming at least one standard alcoholic drink per day. Furthermore, 208 (54.6%) of the study participants were current khat chewers, with 119 (31.2%) of them chewing khat < 300 g per day (Table 2).

Admission Diagnosis of the Patients

Of 381 patients admitted to the selected hospitals, 90 (23.6%) were admitted with CKD, followed by chronic liver disease (CLD), 59 (15.5%), and chronic obstructive pulmonary disease (COPD) (13.9%) (Figure 2).

Prevalence of Anemia Among Admitted Patients

The mean hemoglobin level of the study participants was 10.85 g/dl $\pm$ 3.5 and ranged from 4.0 to 18.0 g/dl. The overall prevalence of anemia among admitted patients was 64.8% (CI: 60.01, 69.65). Out of this prevalence, the most common

**Figure 2** Admission diagnosis of hospital admitted patients in Eastern Ethiopia, 2022.

Abbreviation: NCD, Non-Communicable Disease.

causes of anemia were CKD (31.2%), followed by CLD (14.6%) and infectious disease (13.4%). The least common cause of anemia in the current study was pneumonia, with a prevalence of 2.8% (Figure 3).

The Severity of Anemia Among Admitted Patients

Out of 247 (64.8%) anemic patients, 54 (14.2%) had mild anemia, 132 (34.6%) had moderate anemia, and 61 (16.0%) had severe anemia. In terms of anemia severity, the highest number of mild anemias was observed in participants admitted with CKD (31.5%), followed by CLD (18.4%). On the other hand, subjects admitted with chronic NCD (3.7%) showed the least mild anemia. In terms of moderate anemia, patients admitted with CKD had the highest percentage of cases (35.8%), followed by infectious diseases and deep vein thrombosis (15.2% of each). However, those admitted with inflammatory bowel disease (IBD) (1.5%) had the least amount of moderate anemia. In terms of severe anemia, patients admitted with CKD and CLD (23.0% of each) had the greatest percentages of severe anemia. However, admitted patients with pneumonia did not exhibit severe anemia (Figure 4).

Morphological Types of Anemia Among Admitted Patients

Based on MCV and MCHC, normocytic normochromic anemia constituted the majority of anemia (33.2%), followed by microcytic hypochromic anemia (27.9%). However, macrocytic normochromic anemia had the lowest percentage of anemia (7.8%) (Figure 5).

Factors Associated with Anemia

In the bi-variable logistic regression analysis, educational status, alcohol consumption, BMI, MUAC, and admission diagnosis were significantly associated at a p -value of < 0.25 and considered potential candidates for multivariable logistic regression analysis. In multivariable logistic regression, alcohol consumption, BMI, MUAC, and admission diagnosis were independently associated with greater odds for the presence of anemia at a p -value of < 0.05 . Admitted patients who drank standard alcohol for 1–4 days per week were 2.88 times more likely (AOR = 2.88, 95% CI = 1.45, 5.70) to develop anemia than those who did not drink standard alcohol. Moreover, admitted patients who drank standard

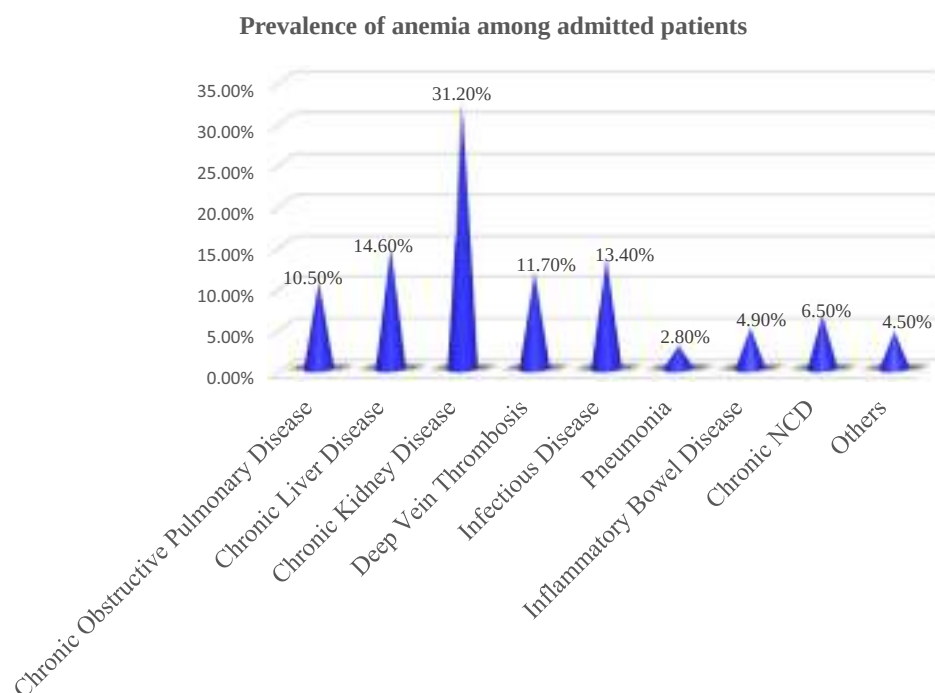


Figure 3 Prevalence of anemia among admission diagnosis of hospital admitted patients in Eastern Ethiopia, 2022.

Abbreviation: NCD, Non-Communicable Disease.

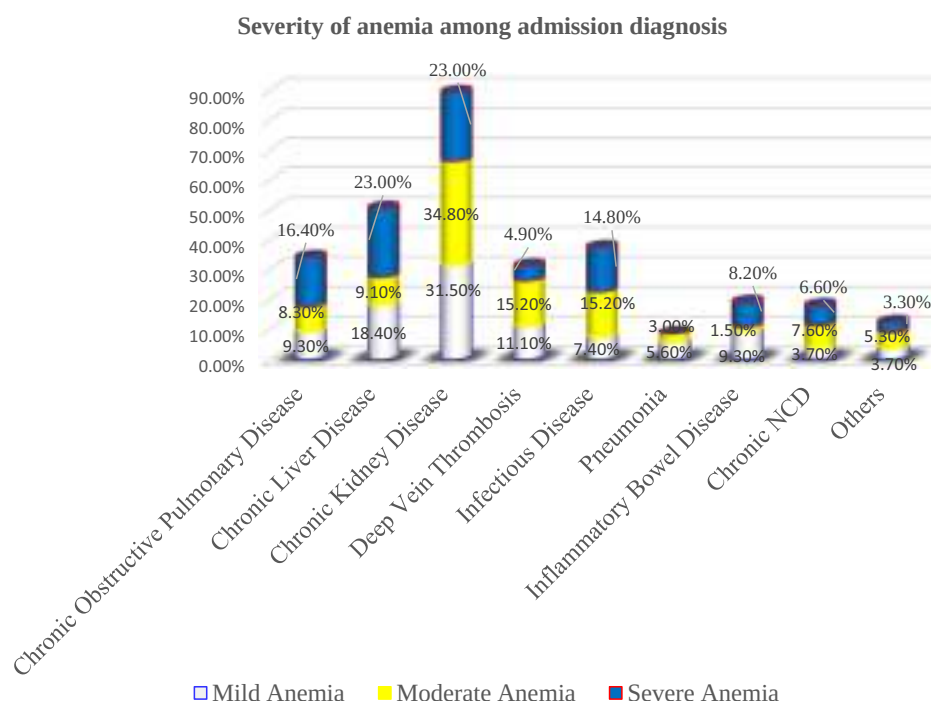


Figure 4 Severity of anemia among admission diagnosis of hospital admitted patients in Eastern Ethiopia, 2022.

Abbreviation: NCD, Non-Communicable Disease

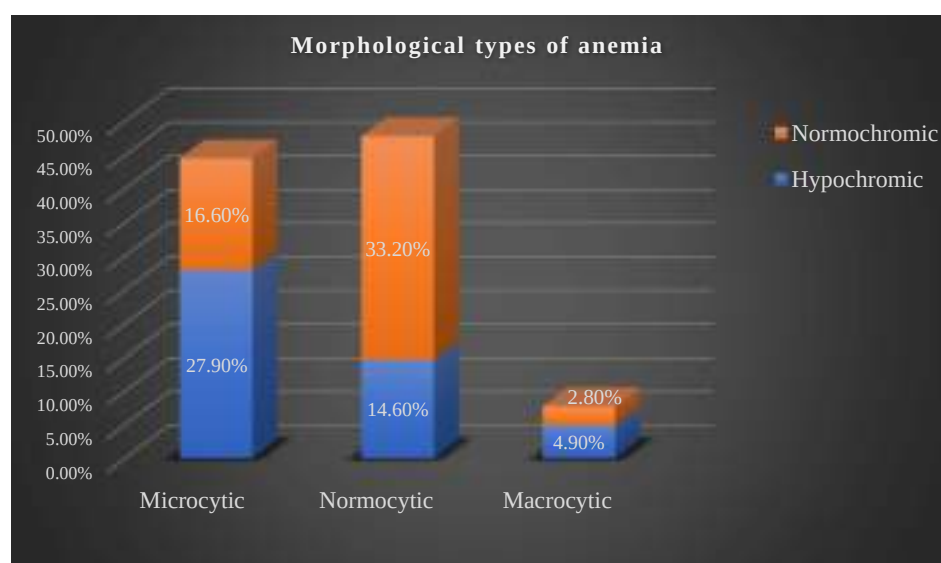


Figure 5 Morphological types of anemia among hospital admitted patients in Eastern Ethiopia, 2022.

alcohol daily were 3.8 times more likely (AOR = 3.78, 95% CI = 1.71, 8.30) to develop anemia than those who did not drink standard alcohol.

Underweight admitted patients were 9.39 times more likely (AOR = 9.39, 95% CI = 2.90, 30.46) to develop anemia than patients who had normal weight. Additionally, obese admitted patients were 2.30 times more likely (AOR = 2.30, 95% CI = 1.00, 5.27) to develop anemia than normal-weight admitted patients. Undernourished admitted patients had 2.59 times higher odds (AOR = 2.59, 95% CI = 1.15, 5.84) of developing anemia than normal admitted patients. Patients admitted with CLD had 3.20 times higher odds (AOR = 3.20, 95% CI = 1.21, 8.47), CKD had 11.2 times higher odds

Table 3 Bivariable and Multivariable Logistic Regression Analysis for the Factors Associated with Anemia Among Hospital Admitted Patients in Eastern Ethiopia, 2022

Variables	Category	Anemia Status		95% CI	
		Yes N (%)	No N (%)	COR	AOR
Age in years	15–24	44 (65.7)	23 (34.3)	1.24 (0.49, 3.08)	1.33 (0.38, 4.71)
	25–34	80 (66.7)	40 (33.3)	1.29 (0.55, 3.02)	1.35 (0.42, 4.39)
	35–44	14 (25.5)	41 (74.5)	1.89 (0.72, 5.01)	1.71 (0.46, 6.29)
	45–54	28 (58.3)	20 (41.7)	0.91 (0.35, 2.35)	0.88 (0.25, 3.17)
	55–64	37 (58.7)	26 (41.3)	0.92 (0.37, 2.29)	0.95 (0.27, 3.34)
	Above 65	17 (60.7)	11 (39.3)	1	1
Sex	Female	131 (66.2)	67 (33.8)	0.89 (0.58, 1.35)	0.91 (0.51, 1.62)
	Male	116 (63.4)	67 (36.6)	1	1
Residence	Urban	113 (66.1)	58 (33.9)	1.11 (0.72, 1.69)	1.09 (0.62, 1.93)
	Rural	134 (63.8)	76 (36.2)	1	1
Educational Status	Unable to read and write	53 (70.7)	22 (29.3)	1.71 (0.86, 3.39)	1.36 (0.54, 3.43)
	Able to read and write	60 (75.0)	20 (25.0)	2.12 (1.06, 4.25)	2.13 (0.86, 5.31)
	Preparatory School	42 (56.8)	32 (43.2)	0.93 (0.48, 1.79)	0.47 (0.19, 1.15)
	Secondary School	51 (62.2)	31 (37.8)	1.16 (0.61, 2.23)	0.81 (0.34, 1.89)
	College or above	41 (58.6)	29 (41.4)	1	1
Amount of standard alcohol drink	No	72 (45.6)	86 (54.4)	1	1
	1– 4 days per a week	63 (70.0)	27 (30.0)	2.79 (1.61, 4.83)	2.88 (1.45, 5.70)*
	Daily	112 (84.2)	21 (15.8)	6.37 (3.63, 11.17)	3.78 (1.72, 8.30)*
BMI	Normal	52 (45.6)	62 (54.4)	1	1
	Underweight	108 (94.7)	6 (5.3)	21.46 (8.72, 52.84)	9.39 (2.90, 30.45)*
	Overweight	54 (57.4)	40 (42.6)	1.61 (0.93, 2.79)	1.61 (0.77, 3.41)
	Obese	33 (55.9)	26 (44.1)	1.51 (0.80, 2.85)	2.30 (1.00, 5.27)*
MUAC	Normal	106 (50.2)	105 (49.8)	1	1
	Undernourished	141 (82.9)	29 (17.1)	4.82 (2.97, 7.80)	2.59 (1.15, 5.83)*
Admission Diagnosis	COPD	26 (49.1)	27 (50.9)	1	1
	CLD	36 (61.0)	23 (39.0)	1.63 (0.77, 3.44)	3.20 (1.21, 8.47)*
	CKD	77 (85.6)	13 (14.4)	6.15 (2.77, 13.65)	11.15 (4.06, 30.60)*
	Deep vein thrombosis	29 (78.4)	8 (21.6)	3.76 (1.46, 9.74)	6.21 (1.98, 19.51)*
	Infectious disease	33 (84.6)	6 (15.4)	5.71 (2.05, 15.89)	9.70 (2.77, 34.02)*
	Pneumonia	7 (20.6)	27 (79.4)	0.27 (0.10, 0.70)	0.61 (0.18, 2.02)
	IBD	12 (42.9)	16 (57.1)	0.78 (0.31, 1.96)	1.29 (0.42, 4.04)
	Chronic NCD	16 (69.6)	7 (30.4)	2.37 (0.84, 6.71)	7.02 (1.90, 25.99)*
	Others	11 (61.1)	7 (38.9)	1.63 (0.55, 4.85)	3.76 (0.95, 14.87)

Note: *Statistically Significance at p value of < 0.05.

Abbreviations: BMI, Body Mass Index; MUAC, Middle Upper Arm Circumference; COPD, Chronic Obstructive Disease; CLD, Chronic Liver Disease; CKD, Chronic Kidney Disease; IBD; Inflammatory Bowel Disease; NCD, Non communicable Disease; COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio.

(AOR = 11.16, 95% CI = 4.06, 30.64), deep vein thrombosis had 6.22 times higher odds (AOR = 6.22, 95% CI = 1.98, 19.52), infectious disease had 9.71 times higher odds (AOR = 9.71, 95% CI = 2.77, 34.02), and chronic NCD had 7.01 times higher odds (AOR = 7.01, 95% CI = 1.90, 25.99) to develop anemia as compared to patients admitted with COPD (Table 3).

Discussion

In this study, the overall prevalence of anemia among hospital admitted patients was 64.80%. Of these, 14.2% had mild anemia, 34.6% had moderate anemia, and 16.0% had severe anemia. This study was concordant with the studies conducted in Germany 60%,²⁸ Benin 61.8%,⁴ and Uganda 64.3%.⁵ However, Results from the present study revealed a lower prevalence of anemia than the previous studies conducted in Pakistan 71%,³ Bahrain 72.2%,² and Tanzania 79.5%,¹¹ and a higher prevalence than other similar studies done in Italy 48%,⁶ Germany 54.2%,²⁹ Pakistan 55.5%,³⁰ and Tanzania 44%.¹² This inconsistency

might be attributed to variations in the study design, sampling techniques and sample size, socio-demographic, behavioral characteristics, type of admission diagnosis, nutritional variation, and the age of the study subjects.

Normocytic normochromic (33.2%) blood picture was the most common morphological type of anemia, followed by microcytic hypochromic (27.9%) anemia. Whereas the least morphological type of anemia was macrocytic normochromic (2.8%) anemia found in this study. It is not surprising to see a normocytic normochromic blood picture of anemia in this study, as various previous studies conducted in India,³¹ Pakistan,³² Malaysia,³³ Saudi Arabia,²² and Benin⁴ also revealed this situation. Since the majority of participants in this study were admitted patients with CKD, chronic NCD, and ACD, which are expected to be normocytic and normochromic in morphology at the early stage of the disease.^{33–35}

Admitted patients who drank standard alcohol for 1–4 days per week and daily were 2.88 (AOR = 2.88, 95% CI = 1.45, 5.70) and 3.78 (AOR = 3.78, 95% CI = 1.71, 8.30) times more likely to develop anemia than those who did not drink standard alcohol, respectively. The result was in agreement with the studies conducted in Taiwan,³⁶ Finland,³⁷ and Japan,³⁸ as heavy drinkers were more likely to develop anemia than non-drinkers. Anemia in alcoholics has a complicated and multifaceted etiology, with causes ranging from poor nutrition to chronic inflammation, blood loss, liver failure, and inefficient erythropoiesis.^{39,40}

Underweight admitted patients had 9.39 (AOR = 9.39, 95% CI = 2.90, 30.46) times higher odds of developing anemia than patients who had normal weight. The result was consistent with the studies conducted in Japan,⁴¹ Israel,⁴² and Tanzania.⁴³ The possible reasons for the underweight might be due to disease-specific factors such as loss of appetite, inflammation, swallowing difficulties, hypercatabolism, and treatment-related factors such as fasting episodes, treatment side effects, and psychological factors (anxiety, depression, and loneliness).^{44,45}

Additionally, obese admitted patients had 2.30 (AOR = 2.30, 95% CI = 1.00, 5.27) times higher odds of developing anemia than patients who had normal weight. The result was in agreement with the studies conducted in the United States of America,⁴⁶ Pakistan,⁴⁷ Iran,⁴⁸ and Salzburg, Austria.⁴⁹ The principal mechanism that links obesity and anemia is low-grade systemic inflammation, which is observed in people with obesity.⁵⁰ In patients with obesity, serum hepcidin, and serum interleukin-6 are significantly higher, which results in reduced iron absorption and sequestration of iron within splenic and hepatic macrophages, which leads to a reduction in iron use in the obese.⁵¹

Undernourished admitted patients had 2.59 times higher odds (AOR = 2.59, 95% CI = 1.15, 5.84) of developing anemia than normal admitted patients. The result was in line with the studies conducted in the Netherlands,⁴⁴ and India.⁵² Malnutrition is associated with poor tolerance to treatment, decreased quality of life, increased health care costs, social factors (such as poverty), and clinical conditions like malabsorption syndrome, intestinal obstruction, and gastric atony.⁵³

Patients admitted with CLD were 3.20 (AOR = 3.20, 95% CI = 1.21, 8.47) times more likely to develop anemia than COPD patients. This finding was supported by researchs undertaken in the United States of America,⁵⁴ India,⁵⁵ and Bangladesh.⁵⁶ This could be due to the following factors: Hemorrhage, particularly in the gastrointestinal tract, is a prominent cause of anemia in people with chronic liver disease and cirrhosis.⁵⁶ Anemia owing to bleeding can arise as a consequence of chronic liver illness due to a deficiency of one or more blood clotting factors produced by the liver, thrombocytopenia, and/or impaired platelet activity.⁵⁷ Furthermore, iron insufficiency is common in late CLD patients because the liver secretes hepcidin, the major regulator of iron homeostasis.⁵⁸

Admitted patients with CKD were 11.2 times (AOR = 11.16, 95% CI = 4.06, 30.64) more likely to develop anemia than COPD patients. Studies conducted in the United States,⁵⁹ Korea,⁶⁰ Indonesia,⁶¹ Peshawar, Pakistan,⁶² and Ghana⁶³ all corroborated this finding. This could be because people with CKD are more likely to develop anemia due to decreased erythropoietin production by failing kidneys. This might be since patients with CKD have a higher risk of developing anemia associated with decreased production of erythropoietin by failing kidneys.⁶⁴ Chronic kidney disease causes anemia by reducing erythropoietin production by the kidney's peritubular fibroblasts resulting in normocytic normochromic anemia, which was found in nearly half of CKD patients.⁶⁵

Admitted deep vein thrombosis patients were 6.22 times (AOR = 6.22, 95% CI = 1.98, 19.52) more likely to develop anemia than COPD patients. This finding was reinforced by researchs undertaken across different corners of China,^{66–68} France,⁶⁹ and Senegal.⁷⁰ The erythropoietin reaction to changes in Hgb levels is linearly logarithmic, which means that the lower the Hgb level, the higher the erythropoietin response.⁷¹ Erythropoietin can promote hypercoagulability and raise the risk of thrombosis by increasing platelet and RBC levels as well as blood viscosity.⁷²

Admitted patients with infectious illness were 9.7 times (AOR = 9.71, 95% CI = 2.77, 34.02) more likely to develop anemia than patients admitted with COPD. This finding was supported by researches from Iran,⁷³ Cameroon,⁷⁴ and Ethiopia.⁷⁵ The primary factor in the development of malaria-related anemia is increased hemolysis of parasitized RBCs and increased destruction of non-parasitized RBCs.⁷⁶ This could be because hepcidin is elevated during infections, which also contributes to anemia.⁷⁷ Anemia prevalence in HIV-positive individuals increases with the progression of the disease and results in chronic acute phase response, elevated hepcidin, anemia of inflammation, and altered iron metabolism.^{78,79} Anemia among pulmonary TB patients is thought to result from anemia of inflammation, as well as increased blood loss from hemoptysis (blood in sputum), decreased RBC production, and poor appetite and food intake, leading to poor nutrient status.⁸⁰

Admitted patients with chronic NCDs were found to be 7.01 times (AOR = 7.01, 95% CI = 1.90, 25.99) more likely to be anemic than patients admitted with COPD. This result was in line with studies conducted in Malaysia,⁸¹ India,⁸² Pakistan,⁸³ and Ghana.²¹ The pathogenesis of anemia in hematological malignant patients is largely speculative. In solid cancers, the most likely cause may be RBC fragmentation in small vessels of cancerous tissue, particularly in bone marrow, the lung, or other organs. Tumor cell cytokine production may also play a role in tumor necrosis factor (TNF)-mediated red cell damage⁸⁴ that activates the coagulation cascade and leads to RBC consumption via thrombotic microangiopathies.⁸²

Conclusion

In this study, 64.80% of hospital admitted patients had anemia, indicating a need for routine screening for all admitted patients.

Abbreviations

ACD, Anemia of Chronic Disease; BMI, Body Mass Index; CKD, Chronic kidney disease; CLD, Chronic Liver Disease; COPD, Chronic Obstructive Pulmonary Disease; HIV, Human Immune Virus; Hgb, Hemoglobin; HFCSUH, Hiwot Fana Comprehensive Specialized University Hospital; IBD, Inflammatory Bowel Disease; IDA, Iron Deficiency Anemia; MCH, Mean Cell Hemoglobin; MCHC, Mean cell Hemoglobin concentration; MCV, Mean Cell Volume; MUAC, Middle-Upper Arm Circumference; NCD, Non-Communicable Disease; RBC, Red Blood Cell; WHO, World Health Organization.

Acknowledgments

The authors would like to thank Haramaya University, all hospital staff members of the hospitals, data collectors, supervisors, study participants, and questionnaire translators for their assistance.

Author Contributions

All authors contributed significantly to the work reported in the conceptualization, study design, execution, acquisition, analysis, and interpretation of data, as well as took part in drafting, revising, and critically assessing the paper. All authors have agreed to approve the final paper for publication in the current journal and to be held accountable for all elements of the work.

Funding

This study got no specific financing from government, commercial, or non-profit organizations.

Disclosure

The authors declare that they have no competing interests.

References

1. Fonseca C, Araujo M, Moniz P, et al. Prevalence and prognostic impact of anemia and iron deficiency in patients hospitalized in an internal medicine ward: the Pro-Iron study. *Eur J Haematol*. 2017;99(6):505–513. doi:10.1111/ejh.12963
2. Alsaed M, Ahmed S, Seyadi K, Ahmed A, Alawi S, Abulsaad K. The prevalence and impact of anemia in hospitalized older adults: a single center experience from Bahrain. *J Taibah Univer Med Sci*. 2022;17(4):587–595. doi:10.1016/j.jtumed.2022.02.003
3. Bashir F, Nageen A, Kidwai S, Zulfikar S, Shiraz S, Ara J. Anemia in hospitalized patient: prevalence, etiology and risk factors. *J Liaq Univer Med Health Sci*. 2017;16:2.

4. Dovonou C, Alassani A, Attinsounon C, et al. Epidemiology of anemia at the internal medicine department in Borgou Departmental Hospital Center (DHC) in Parakou (Benin). *Open J Inter Med*. 2018;8(2):123–130. doi:10.4236/ojim.2018.82013
5. Kuule J, Seremba E, Freers J. Anaemia among patients with congestive cardiac failure in Uganda—its impact on treatment outcomes. *South Af Med J*. 2009;99(12):876–880.
6. Migone De Amicis M, Poggiali E, Motta I, et al. Anemia in elderly hospitalized patients: prevalence and clinical impact. *Intern Emerg Med*. 2015;10:581–586. doi:10.1007/s11739-015-1197-5
7. World Health Organization. *Nutritional Anaemias: Tools for Effective Prevention and Control*. World Health Organization; 2017.
8. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. In: *Vitamin and Mineral Nutrition Information System*. Geneva: World Health Organization; 2011.
9. Halawi R, Moukhadder H, Taher A. Anemia in the elderly: a consequence of aging? *Expert Rev Hematol*. 2017;10(4):327–335. doi:10.1080/17474086.2017.1285695
10. Kurniali P, Curry S, Brennan K, et al. A retrospective study investigating the incidence and predisposing factors of hospital-acquired anemia. *Anemia*. 2014;2014:1–6. doi:10.1155/2014/634582
11. Chamba C, Nasser A, Mawalla W, et al. Anaemia in the hospitalized elderly in Tanzania: prevalence, Severity, and micronutrient deficiency status. *Anemia*. 2021;2021:1–6. doi:10.1155/2021/9523836
12. Munenguni E, Huda A, Jaffer T, Zimbeiya A, Henke O. Anemia in hospitalized patients in A referral hospital in Northern Tanzania. *East Afr Med J*. 2021;98:5.
13. Kassebaum N, Collaborators GA. The global burden of anemia. *Hematol Oncol Clin North Am*. 2016;30(2):247–308. doi:10.1016/j.hoc.2015.11.002
14. Randi M, Bertozzi I, Santarossa C, et al. Prevalence and causes of anemia in hospitalized patients: impact on diseases outcome. *J Clin Med*. 2020;9(4):950. doi:10.3390/jcm9040950
15. Girelli D, Marchi G, Camaschella C. Anemia in the elderly. *HemaSphere*. 2018;2:e40. doi:10.1097/HS9.0000000000000040
16. Weiss G, Goodnough LT. Anemia of chronic disease. *New England J Med*. 2005;352(10):1011–1023. doi:10.1056/NEJMra041809
17. Busti F, Marchi G, Ugolini S, Castagna A, Girelli D. Anemia and iron deficiency in cancer patients: role of iron replacement therapy. *Pharmaceuticals*. 2018;11(4):94. doi:10.3390/ph11040094
18. Camaschella C. Iron-deficiency anemia. *New England J Med*. 2015;372(19):1832–1843. doi:10.1056/NEJMra1401038
19. Bekele A, Teji K, Egata G, Gebremichael B. Anemia and associated factors among type-2 diabetes mellitus patients attending public hospitals in Harari Region, Eastern Ethiopia. *PLoS One*. 2019;14(12):e0225725. doi:10.1371/journal.pone.0225725
20. Wassie M, Aemro A, Fentie B, Khatami F. Prevalence and associated factors of baseline anemia among cervical cancer patients in Tikur Anbesa Specialized Hospital, Ethiopia. *BMC Women's Health*. 2021;21(1):1–8. doi:10.1186/s12905-020-01152-w
21. Anderson A. Prevalence of anemia, overweight/obesity, and undiagnosed hypertension and diabetes among residents of selected communities in Ghana. *Int J Chronic Dis*. 2017;2017:1–7. doi:10.1155/2017/7836019
22. Elsayid M, Al-Qahtani A, Alanazi A, Qureshi S. Determination of the most common morphological patterns of anemia among Saudi anemic patients attending King Abdul-aziz Medical City-Riyadh. *Intern J Med Public Health*. 2015;5:4.
23. Pus A, Moriyama M, Uno M, Rahman MM. Identifying factors of obesity in Papua New Guinea: a descriptive study. *Health*. 2016;8(14):1616–1629. doi:10.4236/health.2016.814158
24. Benítez Brito N, Suárez Llanos J, Fuentes Ferrer M, et al. Relationship between mid-upper arm circumference and body mass index in inpatients. *PLoS One*. 2016;11(8):e0160480. doi:10.1371/journal.pone.0160480
25. World Health Organization. *Guidelines on Drawing Blood: Best Practices in Phlebotomy*. World Health Organization; 2010.
26. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. In: *Vitamin and Mineral Nutrition Information System*. Document Reference World Health Organization; 2011.
27. Ahmed K, Danial K, Khurram A, Wasey M, Ahmed M, Jangda Z. To evaluate the renal function deterioration along with other anemia predictors in patients with diabetes mellitus type 2 in Karachi, Pakistan. *Pak J Surg*. 2017;33(2):135–139.
28. Geisel T, Martin J, Schulze B, et al. An etiologic profile of anemia in 405 geriatric patients. *Anemia*. 2014;2014:1–7. doi:10.1155/2014/932486
29. Röhrig G, Klossok W, Becker I, Benzing T, Schulz R. Prevalence of anemia among elderly patients in an emergency room setting. *Eur Geriatr Med*. 2014;5(1):3–7. doi:10.1016/j.eurger.2013.10.008
30. Awan M, Fazal I, Lodhi M, Azhar R, Anwar A, Tariq M. Frequency of iron deficiency anemia in patients of inflammatory bowel disease at Pak Emirates Military Hospital, Rawalpindi. *Pakistan Arme Forces Med J*. 2022;72(SUPPL-2):S168–71. doi:10.51253/pafmj.v72iSUPPL-2.2785
31. Aithal K, Meti K, Jain S. A study of pattern and causes of anaemia in elderly patients admitted at tertiary centre. *Sch J Appl Med Sci*. 2017;5(4D):1483–1486.
32. Ashraf A. Frequency of anaemia in patients presenting to a tertiary care hospital in Lahore, Pakistan. *Medicine*. 2018;4(19):12.
33. Idris I, Tohid H, Muhammad N, et al. Anaemia among primary care patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD): a multicentred cross-sectional study. *BMJ open*. 2018;8(12):e025125. doi:10.1136/bmjopen-2018-025125
34. Abdulqadir A, Polus R. Prevalence of anemia of chronic disease and iron deficiency anemia among adult diabetic patients in Erbil City. *Zanco J Med Sci*. 2014;18:1.
35. Madu AJ, Ughasoro MD. Anaemia of chronic disease: an in-depth review. *Med Princ Pract*. 2017;26(1):1–9. doi:10.1159/000452104
36. Paramastri R, Hsu C, Lee H, Lin L, Kurniawan A, Chao J. Association between dietary pattern, lifestyle, anthropometric status, and anemia-related biomarkers among adults: a population-based study from 2001 to 2015. *Int J Environ Res Public Health*. 2021;18(7):3438. doi:10.3390/ijerph18073438
37. Latvala J, Parkkila S, Niemelä O. Excess alcohol consumption is common in patients with cytopenia: studies in blood and bone marrow cells. *Alcohol Clin Exp Res*. 2004;28(4):619–624. doi:10.1097/01.ALC.0000122766.54544.3B
38. Yokoyama A, Yokoyama T, Brooks P, et al. Macrocytosis, macrocytic anemia, and genetic polymorphisms of alcohol dehydrogenase-1 b and aldehyde dehydrogenase-2 in Japanese Alcoholic Men. *Alcohol Clin Exp Res*. 2014;38(5):1237–1246. doi:10.1111/acer.12372
39. Colman N, Herbert V. Hematologic complications of alcoholism: overview. *Semin Hematol*. 1980;17(3):164–176.
40. Lewis G, Wise M, Poynton C, Godkin A. A case of persistent anemia and alcohol abuse. *Nat Clin Pract Gastroenterol Hepatol*. 2007;4(9):521–526. doi:10.1038/ncpgasthep0922

41. Kubo S, Hosomi N, Hara N, et al. Ischemic stroke mortality is more strongly associated with anemia on admission than with underweight status. *J Stroke Cerebrov Dis.* 2017;26(6):1369–1374. doi:10.1016/j.jstrokecerebrovasdis.2017.02.016
42. Aronson D, Nassar M, Goldberg T, Kapeliovich M, Hammerman H, Azzam Z. The impact of body mass index on clinical outcomes after acute myocardial infarction. *Int J Cardiol.* 2010;145(3):476–480. doi:10.1016/j.ijcard.2009.12.029
43. Cox S, Makani J, Fulford A, et al. Nutritional status, hospitalization and mortality among patients with sickle cell anemia in Tanzania. *haematologica.* 2011;96(7):948. doi:10.3324/haematol.2010.028167
44. Loh K, Vriens M, Gerritsen A, et al. Unintentional weight loss is the most important indicator of malnutrition among surgical cancer patients. *Neth J Med.* 2012;70(8):365–369.
45. Mariette C, De Botton M-L, Piessen G. Surgery in esophageal and gastric cancer patients: what is the role for nutrition support in your daily practice? *Ann Surg Oncol.* 2012;19:2128–2134. doi:10.1245/s10434-012-2225-6
46. Yanoff L, Menzie C, Denking B, et al. Inflammation and iron deficiency in the hypoferrremia of obesity. *Int J Obes.* 2007;31(9):1412–1419. doi:10.1038/sj.ijo.0803625
47. Khan A, Khan W, Ayub M, Humayun M, Haroon M. Ferritin is a marker of inflammation rather than iron deficiency in overweight and obese people. *J Obes.* 2016;2016:1–7. doi:10.1155/2016/1937320
48. Arshad M, Jabberian S, Pazouki A, Riaz S, Rangraz M, Mokhber S. Iron deficiency anemia and megaloblastic anemia in obese patients. *Rom J Intern Med.* 2017;55(1):3–7. doi:10.1515/rjim-2016-0046
49. Aigner E, Feldman A, Datz C. Obesity as an emerging risk factor for iron deficiency. *Nutrients.* 2014;6(9):3587–3600. doi:10.3390/nu6093587
50. Sal E, Yenicesu I, Celik N, et al. Relationship between obesity and iron deficiency anemia: is there a role of hepcidin? *Hematology.* 2018;23(8):542–548. doi:10.1080/10245332.2018.1423671
51. Saad R, Qutob H. The relationship between anemia and obesity. *Expert Rev Hematol.* 2022;15(10):911–926. doi:10.1080/17474086.2022.2131521
52. Thakur N, Chandra J, Pemde H, Singh V. Anemia in severe acute malnutrition. *Nutrition.* 2014;30(4):440–442. doi:10.1016/j.nut.2013.09.011
53. van Bokhorst-de van der Schueren M. Nutritional support strategies for malnourished cancer patients. *Europ J Oncol Nurs.* 2005;9:S74–S83.
54. Tseng F, Gou G, Wang S, Shyu J, Pan R. Chronic liver disease and cirrhosis increase morbidity in geriatric patients treated surgically for Hip fractures: analysis of the US Nationwide Inpatient Sample. *BMC Geriatr.* 2022;22(1):150. doi:10.1186/s12877-022-02832-y
55. Rauf A, Gupta V, Bansal R, et al. Anemia profile in patients of chronic liver disease from North Indian tertiary hospital. *J Clin Exp Hepatol.* 2014;4:S41–S2.
56. Nuruddin A, Sultana A, Akter F, Ahmed E, Dutta B. Prevalence and pattern of anemia among the hospitalized patients of chronic liver disease in a tertiary care hospital. *J Chittagong Med Coll Teach Assoc.* 2017;28(2):111–115. doi:10.3329/jcmcta.v28i2.62433
57. Farrell G, Larter C. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology.* 2006;43(S1):S99–S112. doi:10.1002/hep.20973
58. Gkamprela E, Deutsch M, Pectasides D. Iron deficiency anemia in chronic liver disease: etiopathogenesis, diagnosis and treatment. *Anna Gastroenterol.* 2017;30(4):405. doi:10.20524/aog.2017.0152
59. Stauffer M, Fan T. Prevalence of anemia in chronic kidney disease in the United States. *PLoS One.* 2014;9(1). doi:10.1371/journal.pone.0084943
60. Lee Y, Chang Y, Kang J, et al. Risk factors for incident anemia of chronic diseases: a cohort study. *PLoS One.* 2019;14:5.
61. Suega K, Bakta M, Dharmayudha TG, Lukman J, Suwitra K. Profile of anemia in chronic renal failure patients: comparison between predialyzed and dialyzed patients at the division of nephrology, department of internal medicine, Sanglah Hospital, Denpasar, Bali, Indonesia. *Dep Intern Med Sanglah Hosp Denpasar Bali Indonesia Inflam.* 2005;1:6–9.
62. Khan A, Afridi M, Ali G, Idrees M, Waqas M. Prevalence of anemia in chronic kidney disease patients in Lady Reading Hospital, Peshawar. *Khyber J Med Sci.* 2018;11:439–442.
63. Amoako Y, Laryea D, Bedu-Addo G, Andoh H, Awuku Y. Clinical and demographic characteristics of chronic kidney disease patients in a tertiary facility in Ghana. *Pan Afr Med J.* 2014;2014:18.
64. Donnelly S. Why is erythropoietin made in the kidney? The kidney functions as a critmeter. *Am J Kid Dis.* 2001;38(2):415–425. doi:10.1053/ajkd.2001.26111
65. Ryu S, Park S, Jung J, et al. The prevalence and management of anemia in chronic kidney disease patients: result from the Korean cohort study for outcomes in patients with chronic kidney disease (KNOW-CKD). *J Korean Med Sci.* 2017;32(2):249–256. doi:10.3346/jkms.2017.32.2.249
66. Feng L, Xu L, Yuan W, Xu Z, Feng Z, Zhang H. Preoperative anemia and total hospitalization time are the independent factors of preoperative deep venous thromboembolism in Chinese elderly undergoing Hip surgery. *BMC Anesthesiol.* 2020;20:1–6. doi:10.1186/s12871-020-00983-2
67. Xiong X, Xu S, Li T, Cheng B. Correlation of the severity of anemia in patients undergoing total joint arthroplasty with preoperative deep vein thrombosis: a retrospective cohort study. *J Orthop Surg Res.* 2022;17(1):1–9. doi:10.1186/s13018-021-02689-8
68. Hung S-H, Lin H-C, Chung S-D. Association between venous thromboembolism and iron-deficiency anemia: a population-based study. *Blood Coagul Fibrinol.* 2015;26(4):368–372. doi:10.1097/MBC.0000000000000249
69. Lecouffe-Desprets M, Néel A, Graveleau J, et al. Venous thromboembolism related to warm autoimmune hemolytic anemia: a case-control study. *Autoimmun Rev.* 2015;14(11):1023–1028. doi:10.1016/j.autrev.2015.07.001
70. Fall A, Proulle V, Sail A, et al. Risk factors for thrombosis in an African population. *Clin Med Insig.* 2014;7:S13401.
71. Goodnough L, Price T, Parvin C, et al. Erythropoietin response to anaemia is not altered by surgery or recombinant human erythropoietin therapy. *Br J Haematol.* 1994;87(4):695–699. doi:10.1111/j.1365-2141.1994.tb06725.x
72. Lippi G, Franchini M, Favaloro E. Thrombotic complications of erythropoiesis-stimulating agents. In: *Seminars in Thrombosis and Hemostasis.* © Thieme Medical Publishers; 2010.
73. Meidani M, Rezaei F, Maracy M, Avijgan M, Tayeri K. Prevalence, severity, and related factors of anemia in HIV/AIDS patients. *J Res Med Sci.* 2012;17(2):138.
74. Bate A, Kimbi H, Lum E, et al. Malaria infection and anaemia in HIV-infected children in Mutengene, Southwest Cameroon: a cross sectional study. *BMC Infect Dis.* 2016;16(1):1–9. doi:10.1186/s12879-016-1853-z
75. Sahle T, Yemane T, Gedefaw L. Effect of malaria infection on hematological profiles of people living with human immunodeficiency virus in Gambella, southwest Ethiopia. *BMC Hematol.* 2017;17:1–8. doi:10.1186/s12878-017-0072-1
76. White N. Anaemia and malaria. *Malar J.* 2018;17(1):1–17.
77. Spottiswoode N, Duffy P, Drakesmith H. Iron, anemia and hepcidin in malaria. *Front Pharmacol.* 2014;5:125. doi:10.3389/fphar.2014.00125

78. Minchella P, Armitage A, Darboe B, et al. Elevated hepcidin is part of a complex relation that links mortality with iron homeostasis and anemia in men and women with HIV infection. *J Nutr*. 2015;145(6):1194–1201. doi:10.3945/jn.114.203158
79. Redig A, Berliner N. Pathogenesis and clinical implications of HIV-related anemia in 2013. *Hematology*. 2013;2013(1):377–381. doi:10.1182/asheducation-2013.1.377
80. Papatheakis P, Piwoz E. Nutrition and Tuberculosis: a review of the literature and considerations for TB control programs. United States agency for international development. *Africa's Health*. 2010;2008:1.
81. Krishnapillai A, Omar M, Ariaratnam S, et al. The prevalence of anemia and its associated factors among older persons: findings from the National Health and Morbidity Survey (NHMS) 2015. *Int J Environ Res Public Health*. 2022;19(9):4983. doi:10.3390/ijerph19094983
82. Gaspar B, Sharma P, Das R. Anemia in malignancies: pathogenetic and diagnostic considerations. *Hematology*. 2015;20(1):18–25. doi:10.1179/1607845414Y.0000000161
83. Burney S, Farooq M, Alvi A. Anemia in elderly hospitalized patients: frequency and association with comorbidities 1 2 3. *J Islamic Inter Med Coll*. 2018;13(4):194–199.
84. Lechner K, Obermeier H. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. *Medicine*. 2012;91(4):195–205. doi:10.1097/MD.0b013e3182603598

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Deferasirox in Patients with Chronic Kidney Disease: Assessing the Potential Benefits and Challenges

Abdulqadir J Nashwan & Mohamed A Yassin

To cite this article: Abdulqadir J Nashwan & Mohamed A Yassin (2023) Deferasirox in Patients with Chronic Kidney Disease: Assessing the Potential Benefits and Challenges, Journal of Blood Medicine, , 589-594, DOI: [10.2147/JBM.S415604](https://doi.org/10.2147/JBM.S415604)

To link to this article: <https://doi.org/10.2147/JBM.S415604>



© 2023 Nashwan and Yassin.



Published online: 27 Nov 2023.



Submit your article to this journal



Article views: 207



View related articles



View Crossmark data



Citing articles: 4 View citing articles

Deferasirox in Patients with Chronic Kidney Disease: Assessing the Potential Benefits and Challenges

Abdulqadir J Nashwan ¹, Mohamed A Yassin ²

¹Nursing Department, Hamad Medical Corporation, Doha, Qatar; ²Department of Medical Oncology/Hematology, National Center for Cancer Care and Research, Hamad Medical Corporation, Doha, Qatar

Correspondence: Abdulqadir J Nashwan, Nursing Department, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar, Tel +974 40240487; +974 66473549, Email anashwan@hamad.qa

Abstract: Chronic kidney disease (CKD) is a major global health concern, affecting millions of people worldwide. The progressive decline in kidney function often necessitates renal replacement therapy, such as hemodialysis (HD) or peritoneal dialysis (PD), to maintain a patient's health. Iron overload, which is common in CKD patients on dialysis, can lead to severe complications, including cardiovascular disease and infections where most of the existing iron chelators are deemed unsuitable due to their suboptimal clearance in patients with compromised renal function, it becomes a significant challenge to effectively manage iron overload. Deferasirox (DFX), an oral iron chelator, has emerged as a promising treatment option for managing iron overload in these patients. However, the use of DFX comes with its unique set of challenges, such as its cost, potential side effects, and the need for close monitoring of patients, as well as the noticeable scarcity of comprehensive and rigorous clinical studies confirming its efficacy and safety of DFX. In this review, we delve into both the promising prospects and the emerging challenges associated with DFX use in managing CKD patients on HD or PD, striving for a comprehensive understanding that informs better clinical practice and patient care.

Keywords: CHRONIC kidney disease, Deferasirox, chelating therapy, iron overload, hemodialysis, peritoneal dialysis

Introduction & Background

Chronic kidney disease (CKD) is a major public health concern which affects around 10% of the global population and is the 6th fastest growing cause of death worldwide.¹ In the United States, approximately 37 million adults, or 15% of the population, have CKD.¹

In the United States, around 88% of patients with end-stage renal disease (ESRD) are treated with hemodialysis (HD), while 12% are treated with peritoneal dialysis (PD).² Globally, HD is the most used renal replacement therapy, with approximately 3.4 million patients receiving treatment, while PD is used by approximately 11% of the ESRD population.²

Iron overload is a common complication in patients with CKD, particularly those on HD or PD.³ It is associated with increased risk of cardiovascular disease, infections, and other complications. A recent systematic review and meta-analysis⁴ estimated the pooled prevalence of severe and mild to moderate hepatic iron overload quantified by MRI was 0.23 and 0.52, respectively. The review revealed a high prevalence of severe hepatic iron overload in patients with ESRD treated by HD.⁴

There has been some controversy over the use of DFX in CKD patients due to concerns over its safety and efficacy. While DFX has been shown to effectively reduce iron overload in CKD patients, its use in this population is still not fully understood. Some studies have suggested that DFX may increase the risk of renal impairment, while others have found no significant adverse effects on kidney function. Additionally, there have been concerns over the potential interactions between DFX and other medications commonly used in CKD patients. There is also some controversy over the optimal

dosing of DFX in CKD patients, with some studies suggesting that lower doses may be safer and more effective than higher doses. Therefore, this review highlights the potential benefits and challenges associated with the use of DFX in patients with CKD on HD or PD.

Review

Potential Benefits

Efficacy in Reducing Iron Overload

Deferasirox (DFX) has demonstrated efficacy in reducing iron overload or hemosiderosis in various clinical settings, including in patients with transfusion-dependent thalassemia and sickle cell disease.⁵ Its mechanism of action involves binding to excess iron and promoting its excretion in the feces, thereby reducing the risk of iron-related complications.⁶ This potential to mitigate iron overload makes DFX an attractive treatment option for CKD patients on dialysis, who often receive frequent blood transfusions and intravenous iron therapy to manage anemia.⁶

Oral Administration

One of the key advantages of DFX is its oral formulation, which offers patients a more convenient alternative to parenteral iron chelators, such as deferoxamine.⁷ Oral administration may improve patient adherence and enhance their overall quality of life.⁷ Moreover, the once-daily dosing regimen of DFX is an added benefit, further simplifying the treatment process for patients on dialysis.⁷

Preservation of Kidney Function

Emerging evidence suggests that DFX may have nephroprotective effects in animal models.⁸ Iron chelation therapy with DFX has been associated with improved kidney function in patients with transfusion-dependent anemias.⁹ Although the exact mechanisms are not fully understood, DFX's ability to reduce iron deposition in the kidneys may contribute to preserving renal function.⁹ Consequently, DFX may offer additional benefits for CKD patients on dialysis by slowing the progression of renal decline.

Challenges and Concerns

Limited Data in CKD Patients on Dialysis

Despite the potential benefits of DFX, there is a limited amount of data regarding its use in patients with CKD on HD or PD. Most studies have focused on patients with transfusion-dependent anemias, and the evidence for DFX in CKD patients remains scarce. Therefore, more research is needed to confirm the safety and efficacy of DFX in this population, as well as to establish appropriate dosing guidelines.

Maker et al¹⁰ evaluated the safety and pharmacokinetics of DFX, an iron chelator, in eight hemodialysis-dependent patients with chronic kidney disease (CKD) who were receiving intravenous iron therapy. Two doses of DFX were administered (10 mg/kg and 15 mg/kg) either acutely or at steady state.¹⁰ The results showed that a dose of 10 mg/kg was insufficient to achieve therapeutic plasma concentrations, while 15 mg/kg-maintained plasma concentrations well above the therapeutic range.¹⁰ However, the observed plasma concentration was higher than expected for this dose, highlighting the importance of profiling drugs in specific patient groups such as CKD with iron overload.¹⁰ No adverse clinical events were observed in the study.¹⁰

On the other hand, Chen et al¹¹ evaluated the efficacy, safety, and tolerability of DFX, an iron chelating agent, in eight HD patients with iron overload caused by transfusion-induced renal anemia. DFX treatment (15 mg/kg/day) caused significant reductions in transferrin saturation (TSAT) and ferritin levels and increases in unsaturated and total iron-binding capacity.¹¹ Although there were no significant changes in hematocrit or erythropoietin levels, monthly transfusion volumes decreased significantly.¹¹ DFX was generally well-tolerated, with common adverse effects being nausea, vomiting, diarrhea, and abdominal pain.¹¹ The study concludes that DFX is effective, safe, and tolerable in improving iron metabolism in HD patients with iron overload.

Moreover, Yii et al¹² reported a case of 54-year-old male with b-thalassemia major and end-stage renal disease (ESRD) was managed with continuous ambulatory peritoneal dialysis (CAPD) and required management of iron overload despite not being able to receive transfusions. DFX was administered orally at a dose of 1000 mg/day, and a total of 12.7L of peritoneal dialysate was collected over a 24-hour period with low levels of iron seen in the fluid.¹² Over a 6-month period, serum ferritin levels decreased from 3869µg/l to 1545µg/l, and there were no episodes of peritonitis.¹² These findings suggest that DFX may be a safe and effective agent for iron removal in iron-overloaded CAPD patients with relatively low dialysate iron levels, which reduces the risk of iron-dependent infections.

Another case reported a patient with ESRD secondary to sickle cell nephropathy who developed recurrent symptomatic hypocalcemia while receiving DFX therapy for iron overload from long-term blood transfusions.¹³ As per the authors, this is the first reported case of this complication with DFX therapy in a patient with ESRD, highlighting the need for caution and close monitoring of electrolyte levels in such patients.¹³

Furthermore, patients with transfusion-dependent thalassemia and ESRD are at risk of iron overload, which can be managed by iron chelating agents such as DFX. A report by Beydoun et al¹⁴ described two thalassemia patients on hemodialysis who were treated with DFX, resulting in a decrease in ferritin levels without any significant adverse events except for asymptomatic hypocalcemia in one patient. The dose used (up to 25 mg/kg/day) was higher than previously reported, indicating that DFX may be safe and effective for iron overload management in thalassemia patients with ESRD.¹⁴ Further studies are needed to confirm these findings.

A recent study by Bekhechi et al¹⁵ evaluated the efficacy of DFX on reducing liver iron concentration (LIC) in 31 dialysis patients (30 HD, and 1 PD) with secondary hemosiderosis quantified by magnetic resonance imaging (MRI). The chelation therapy resulted in a significant reduction in LIC, mean ferritin level, and a gain in mean hemoglobin and albumin levels.¹⁵ The therapeutic response was influenced by the cause and degree of overload.¹⁵ These findings suggest that DFX, prescribed at a dose of 10 mg/kg/day, can be effective in reducing iron overload in dialysis patients and may have additional benefits in improving anemia and hypoalbuminemia.¹⁵

Table 1 presents a comprehensive overview of significant research findings pertaining to the utilization of DFX in managing patients with CKD who are undergoing HD or PD.

Adverse Effects

The use of DFX is not without potential risks. Common side effects include gastrointestinal symptoms, such as abdominal pain, nausea, and diarrhea. More serious adverse effects, although rare, can include hypocalcemia,^{13,14} renal impairment, hepatic dysfunction, and hematologic abnormalities.¹⁶ These potential complications may be of particular concern in CKD patients on dialysis, who often have multiple comorbidities and are more susceptible to drug-related adverse events.¹⁶ Careful patient monitoring and dose adjustments may be necessary to minimize the risks associated with DFX therapy.

Drug Interactions

DFX is metabolized by the liver and may interact with other medications commonly prescribed to CKD patients, such as phosphate binders, antihypertensive drugs, and erythropoiesis-stimulating agents.¹⁷ These interactions can potentially alter the effectiveness of the medications and increase the risk of adverse effects.¹⁷ Therefore, healthcare providers must carefully consider potential drug interactions and adjust treatment regimens accordingly to optimize patient outcomes.

Cost and Access

The cost of DFX can be a significant barrier to its widespread adoption in CKD patients on dialysis.¹⁸ DFX is generally more expensive than traditional parenteral iron chelators, which may limit its accessibility, particularly for patients in low-resource settings or those without adequate insurance coverage. Efforts to address cost-related concerns and improve access to DFX will be crucial for ensuring that more patients can benefit from this therapy.

Table I Summary of Key Findings on the Use of DFX in Patients with Chronic Kidney Disease on Hemodialysis or Peritoneal Dialysis

Authors	Year of Publication	Country	Study Design	Number of Patients (Age)	HD vs PD	DFX Dosage/Frequency	Main Findings	Adverse Events
Maker et al ¹⁰	2013	Australia	Pilot study (prospective)	8	HD	Two doses tested: 10 mg/kg and 15 mg/kg. Administered either acute (once daily for 2 days) or steady state (once daily for 2 weeks) 15 mg/kg/day	A dose of 10 mg/kg did not achieve a therapeutic plasma concentration, whereas a dose of 15 mg/kg maintained plasma concentration well above the therapeutic range.	No adverse clinical events were observed.
Chen et al ¹¹	2015	Taiwan	Retrospective	8	HD		DFX administration led to significant reductions in TSAT and ferritin and significant increases in UIBC and TIBC. No significant differences were noted in hematocrit values or EPO requirements post-treatment, but significant reductions were observed in average monthly transfusion volumes.	Nausea, vomiting, diarrhea, and abdominal pain.
Yii et al ¹²	2018	Australia	Case report	1 (Age 54; Male)	PD (patient with b-Thalassemia major developed ESRD)	1000 mg/day	Over a 6-month period, the patient's serum ferritin fell from 3869 µg/l to 1545 µg/l. Iron levels in the peritoneal dialysate were lower than expected, suggesting that DFX may be a safe agent for iron removal in iron-overloaded PD patients.	No episodes of peritonitis were reported.
Yusuf et al ¹³	2008	USA	Case report	1 (Age 43; Female)	PD (ESRD secondary to sickle cell nephropathy)	1500 mg/day (corresponding to 20 mg/kg for her weight)	The patient responded well to DFX therapy, with a progressive decrease in serum ferritin levels over several months.	Recurrent symptomatic hypocalcemia leading to periorbital paresthesias and mental slowing. Patient A had an episode of asymptomatic hypocalcemia
Beydoun et al ¹⁴	2016	Lebanon	Letter to the editor with 2 cases	2 (A: 50 y; Male); (B: 59 y; Female)	HD (A: β-thalassemia major); (B: transfusion-dependent β-thalassemia intermedia)	Initially 15 mg/kg/day, gradually increased to 25 mg/kg/day	For patient A, ferritin levels significantly decreased from 3846 to 1447 ng/mL over 11 months. For patient B, ferritin levels showed only a mild improvement initially before increasing again.	
Bekhechi et al ¹⁵	2023	Algeria	Longitudinal Study	31	30 HD PD	10 mg/kg/day	Chelation with DFX resulted in a significant reduction in liver iron burden and ferritin level, as well as an increase in mean hemoglobin and albumin levels. The therapeutic response was influenced by the cause and degree of iron overload.	Nausea, vomiting, diarrhea, and abdominal pain.

Abbreviations: UIBC, Unsaturated Iron-Binding Capacity; TSAT, Transferrin saturation; TIBC, Total Iron Binding Capacity; EPO, Erythropoietin.

Conclusion

DFX represents a promising treatment option for managing iron overload in patients with CKD on hemodialysis or peritoneal dialysis. Its oral administration, potential nephroprotective effects, and demonstrated efficacy in reducing iron overload make it an attractive alternative to traditional parenteral iron chelators. However, several challenges, including limited data in CKD patients on dialysis, potential adverse effects, drug interactions, and cost concerns, must be addressed before DFX can be widely adopted in this population. Future research should focus on evaluating the safety and efficacy of DFX in CKD patients on dialysis, as well as on exploring strategies to mitigate potential risks and improve patient outcomes. Additionally, efforts should be made to increase access to DFX and to develop patient-centered treatment strategies that promote adherence and enhance the overall quality of life for individuals with CKD on dialysis. By addressing these challenges, DFX may ultimately become an integral component of the comprehensive management of iron overload in patients with CKD on HD or PD.

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

The publication of this article was funded by the Qatar National Library.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Carney EF. The impact of chronic kidney disease on global health. *Nat Rev Nephrol*. 2020;16(5):251. doi:10.1038/s41581-020-0268-7
2. Pannu N, Klarenbach S, Wiebe N, Manns B, Tonelli M; Alberta Kidney Disease Network FT. Renal replacement therapy in patients with acute renal failure: a systematic review. *JAMA*. 2008;299(7):793–805. doi:10.1001/jama.299.7.793
3. Nashwan AJ, Yassin MA, Mohamed Ibrahim MI, Abdul Rahim HF, Shraim M. Iron overload in chronic kidney disease: less ferritin, more T2* MRI. *Front Med*. 2022;9:865669. doi:10.3389/fmed.2022.865669
4. Nashwan AJ, Yassin MA, Abd-Alrazaq A, et al. Hepatic and cardiac iron overload quantified by magnetic resonance imaging in patients on hemodialysis: a systematic review and meta-analysis. *Hemodial Int*. 2023;27(1):3–11. doi:10.1111/hdi.13054
5. Taher A, El-Beshlawy A, Elalfy MS, et al. Efficacy and safety of deferasirox, an oral iron chelator, in heavily iron-overloaded patients with β -thalassaemia: the ESCALATOR study. *Eur J Haematol*. 2009;82(6):458–465. doi:10.1111/j.1600-0609.2009.01228.x
6. Choudhry VP, Naithani R. Current status of iron overload and chelation with deferasirox. *Indian J Pediatr*. 2007;74(8):759–764. doi:10.1007/s12098-007-0134-7
7. Shah NR. Advances in iron chelation therapy: transitioning to a new oral formulation. *Drugs Context*. 2017;2017:6.
8. Cappellini MD. Long-term efficacy and safety of deferasirox. *Blood Rev*. 2008;22:S35–41. doi:10.1016/S0268-960X(08)70007-9
9. Sato T, Kobune M, Murase K, et al. Iron chelator deferasirox rescued mice from Fas-induced fulminant hepatitis. *Hepatol Res*. 2011;41(7):660–667. doi:10.1111/j.1872-034X.2011.00821.x
10. Maker GL, Siva B, Batty KT, Trengove RD, Ferrari P, Olynyk JK. Pharmacokinetics and safety of deferasirox in subjects with chronic kidney disease undergoing haemodialysis. *Nephrology*. 2013;18(3):188–193. PMID: 23332024. doi:10.1111/nep.12035
11. Chen CH, Shu KH, Yang Y. Long-term effects of an oral iron chelator, deferasirox, in hemodialysis patients with iron overload. *Hematology*. 2015;20(5):304–310. PMID: 25200910. doi:10.1179/1607845414Y.0000000199
12. Yli E, Doery JC, Kaplan Z, Kerr PG. Use of deferasirox (Exjade) for iron overload in peritoneal dialysis patients. *Nephrology*. 2018;23(9):887–889. PMID: 29663590. doi:10.1111/nep.13389
13. Yusuf B, McPhedran P, Brewster UC. Hypocalcemia in a dialysis patient treated with deferasirox for iron overload. *Am J Kidney Dis*. 2008;52(3):587–590. PMID: 18534729. doi:10.1053/j.ajkd.2008.03.034
14. Beydoun HG, Saliba AN, Taher AT. Deferasirox in thalassemia patients with end-stage renal disease. *Am J Hematol*. 2016;91(10):E456–7. doi:10.1002/ajh.24457

15. Bekhechi W, Chiali H, Khelil L, Sari-Hamidou R, Benmansour M. Hemosiderosis in chronic dialysis patients: monitoring the response to deferasirox by quantitative hepatic magnetic resonance imaging. *Hemodial Int*. 2023;27(3):270–277. doi:10.1111/hdi.13081
16. Díaz-García JD, Gallegos-Villalobos A, Gonzalez-Espinoza L, Sanchez-Nino MD, Villarrubia J, Ortiz A. Deferasirox nephrotoxicity—the knowns and unknowns. *Nat Rev Nephrol*. 2014;10(10):574–586. doi:10.1038/nrneph.2014.121
17. Roemhild K, von Maltzahn F, Weiskirchen R, Knüchel R, von Stillfried S, Lammers T. Iron metabolism: pathophysiology and pharmacology. *Trend Pharmacol Sci*. 2021;42(8):640–656. doi:10.1016/j.tips.2021.05.001
18. Delea TE, Sofrygin O, Thomas SK, Baladi JF, Phatak PD, Coates TD. Cost effectiveness of once-daily oral chelation therapy with deferasirox versus infusional deferoxamine in transfusion-dependent thalassaemia patients: US healthcare system perspective. *Pharmacoeconomics*. 2007;25(4):329–342. doi:10.2165/00019053-200725040-00005

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Patient Blood Management and Its Role in Supporting Blood Supply

Richard R Gammon, Rounak Dubey, Gaurav K Gupta, Colleen Hinrichsen, Aikaj Jindal, Divjot Singh Lamba, Sadhana Mangwana, Amita Radhakrishnan Nair, Shaughn Nalezinski & Christopher Bocquet

To cite this article: Richard R Gammon, Rounak Dubey, Gaurav K Gupta, Colleen Hinrichsen, Aikaj Jindal, Divjot Singh Lamba, Sadhana Mangwana, Amita Radhakrishnan Nair, Shaughn Nalezinski & Christopher Bocquet (2023) Patient Blood Management and Its Role in Supporting Blood Supply, Journal of Blood Medicine, , 595-611, DOI: [10.2147/JBM.S387322](https://doi.org/10.2147/JBM.S387322)

To link to this article: <https://doi.org/10.2147/JBM.S387322>



© 2023 Gammon et al.



Published online: 29 Nov 2023.



Submit your article to this journal



Article views: 637



View related articles



View Crossmark data



Citing articles: 8 View citing articles

Patient Blood Management and Its Role in Supporting Blood Supply

Richard R Gammon^{1*}, Rounak Dubey^{2*}, Gaurav K Gupta^{3*}, Colleen Hinrichsen^{4*}, Aikaj Jindal^{5*}, Divjot Singh Lamba^{6*}, Sadhana Mangwana^{7*}, Amita Radhakrishnan Nair^{8*}, Shaughn Nalezinski^{9*}, Christopher Bocquet¹⁰

¹Scientific, Medical and Technical Department, OneBlood, Orlando, FL, USA; ²Department of Transfusion Medicine, All India Institute of Medical Sciences, Nagpur, India; ³Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA; ⁴Department of Transfusion Medicine, Princeton Medical Center at Penn Medicine, Plainsboro, NJ, USA; ⁵Department of Transfusion Medicine, Mohandas Oswal Hospital, Ludhiana, India; ⁶Department of Transfusion Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India; ⁷Department of Transfusion Medicine and Immunohematology, Sri Balaji Action Medical Institute, New Delhi, India; ⁸Department of Transfusion Medicine Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India; ⁹Department of Laboratory Medicine - Transfusion Services, Concord Hospital, Concord, NH, USA; ¹⁰Association for the Advancement of Blood and Biotherapies, Bethesda, MD, USA

*These authors contributed equally to this work

Correspondence: Richard R Gammon, OneBlood, 8669 Commodity Circle, Orlando, FL, 32819, USA, Tel +1 407 947 7963, Fax +1 407 264 8265, Email richard.gammon@oneblood.org

Abstract: Blood donors and voluntary blood donations are essential for ensuring the blood supply that can be maintained by good patient blood management (PBM) practices. This review article explores the role of blood donation in PBM and highlights the importance of donor screening and selection processes in different regions worldwide. The donor health questionnaires and the focused physical examination guidelines have changed in the last decade to increase donor and recipient safety. This article also discusses the status of transfusion practices, including the challenges of ensuring a safe blood supply. Significant among these are the effects of the COVID-19 pandemic on the blood supply chain and the impact of an aging donor population, especially. Promoting autologous donations and other blood conservation strategies are suggested to mitigate these issues. The role of replacement donors and the upper age limit for voluntary blood donation may be decided based on the demography and donor pool. The involvement of C-suite executives is also critical in implementing and running a successful PBM program. The review highlights how these different aspects of blood donation are integral to a successful PBM program and the safety of patients who receive blood transfusions.

Keywords: donors, blood centers, transfusion service

Introduction

Ensuring that the blood supply is adequate to support the healthcare system and ensure blood is available to meet patient needs is essential.¹ Ten years of declining blood use and changes in healthcare delivery place the current system's sustainability at risk.² Short-lived blood component shortages are common and managed with short-term solutions.³ A long-term shortage requires additional palliation strategies.⁴ Today, the blood donor population is aging (Table 1). Data from the US National Blood Collection and Utilization Survey between 2017 and 2019 showed a decline in all donor age groups with the exception of those 65 and older.⁵ New ways to engage younger generations of blood donors must be found.¹

PBM has grown and has become the accepted standard of care for patients.⁶ This evidence is supported by the rapid growth of publications since 2006.⁶ The global definition of PBM is as follows:

Patient blood management is a patient-centered, systematic, evidence-based approach to improve patient outcomes by managing and preserving a patient's blood while promoting patient safety and empowerment.⁶

PBM involves vein-to-vein safety.

Table I Percent Change in Donors by Age in the US Between 2017 and 2019

Age	% Change 2019–2017
15	31.7
16	−0.3
17	−11.8
18	−16.1
16–18	−10.1
19–24	−15.1
25–64	−1.0
65 and older	10.5
Total Successful Donations	−1.1

Notes: Adapted from Mowla SJ, Sapiano MRP, Jones JM, Berger JJ, Basavaraju S V. Supplemental findings of the 2019 National Blood Collection and Utilization Survey. *Transfusion*. 2021;61(S2):S11–S35. Published 2021. This article is a US Government work and is in the public domain in the USA.⁵

The purpose of this manuscript is three-fold. First, it will review the donation of blood process both in the United States (US) and globally. Second, it will discuss PBM’s importance for patient safety and ensuring the judicious use of blood. Finally, the manuscript shows how having an adequate supply of blood donors and blood donations is essential for ensuring good PBM practice for those patients, even with all of the alternative therapies available, who continue to require blood transfusions to support their care.⁷

Materials and Methods

This review article is part of a thematic series that explores the importance of blood donation and the role of PBM in supporting the blood supply. A panel of experts in PBM and transfusion medicine were invited to contribute to this article. The initial online meeting occurred in October 2022, when the article’s outline was finalized. Each contributing author was assigned specific sections to write based on their respective areas of interest and expertise. The compiled manuscript underwent thorough revisions, edits, and approval by all authors before its final submission.

Selection of Allogeneic Blood Donors

Registration of Blood Donors

Blood donor registration is collecting information from prospective volunteer blood donors. The essential information required for donor registration typically includes personal details, including the donor’s name, gender, date of birth, address, and telephone number. In the blood donor registration process, donors are assigned a unique donor identification number, receive educational material on blood donation, and complete a donor health questionnaire to determine eligibility. Donor health questionnaires are required to be completed for each donation. Donor registration creates a database of potential donors, whom the donor center can contact to replenish the blood supply and determine if a specific blood product is needed for a particular patient.

Educational Materials and Donor Acknowledgement and Consent

The donor selection process begins with public outreach and donor education. This will increase the likelihood of acceptance.⁸ Donors should be allowed to self-defer after being informed about the health issues and high-risk behaviors

that would prevent blood donation.⁸ Strategies to promote voluntary blood donation, including donor education and information, must be practiced to create a secure and long-lasting voluntary donor base.⁹

Pre-donation information should be presented at the donation session in an easy-to-understand manner and in the appropriate languages.⁸ Donors should be educated regarding the donor selection criteria, blood donation process, and screening tests performed. This lets the donors know the importance of disclosing any medical illness or relevant transfusion-transmitted infectious (RTTI)-associated risks and the unsuitability to donate blood and make a choice to self-defer themselves. Pre-donation information should include knowledge about blood and its components, the importance of blood donation, and the consequences of leading a healthy lifestyle.⁸ It should inform the donor regarding the pre-donation questionnaire, medical history, risk assessment, venipuncture, blood collection, post-donation care, and screening tests done. It should provide the donor with a rationale for the donor questionnaire and pre-donation health evaluation. It should inform the donor about the option to withdraw or self-defer at any moment during or after the donation procedure and to do so without being unduly embarrassed or questioned.⁸ The donor should be informed about routes of transmission, natural history, and ways to prevent RTTIs such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), as well as the window period of infection and alternate testing locations for them to know their infection status.⁸ Donors must be informed that when RTTI test results are abnormal, there are implications for donors and the blood they donate. There is also a mechanism for notifying donors of abnormal test results, providing post-donation counseling, assuring confidentiality, and, if necessary, referring donors for additional testing, and medical treatment.⁸

Donors should be educated regarding the possibility of adverse donor reactions as well. Evaluating the capacity to comprehend blood donation and informed consent is essential.⁸ Donors will be comforted that their welfare is vital to the blood center. They may be motivated to become repeat donors if they understand that providing accurate and complete information about their health is in their best interests. Verifying that the donor understands the questionnaire presented and offers precise information is crucial. This is especially important if the donor is a first-time donor. Responding to the donor's inquiries and offering assurance if necessary is essential. A prospective donor must voluntarily agree to the following: blood donation, testing for RTTI; transfusion of donated blood to patients; and, if necessary, using the blood for additional tests, quality assurance, or research.⁸ To obtain informed consent from the donor, they should be informed about the tests (RTTI and others) conducted on the blood samples given, the justifications for these tests, and assured that all personal data, including test results, are treated confidentially. The process includes the donor's signature and providing informed consent. It indicates agreement to permitting post-donation notification and counseling should a positive RTTI marker or any other abnormality be found and that the donor is aware of the risks of RTTI transmission through donated blood.⁸ In countries where donors may face legal consequences for delivering false information, they must comprehend the questionnaire and its implications. In countries where those under the age of majority may donate, a parent or guardian's written consent must be sought per domestic laws.⁸

Health History Assessment- AABB DHQ

A blood center's role is both donor and recipient safety.⁸ A blood donor should be in good health. The WHO defined "Health" as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity".¹⁰ Good health can be determined using specific associated parameters ascertained from a brief medical history, observation, and simple tests.⁸ The donor assessment aims to determine suitability so that the donation is safe and the products will not harm the recipients. This provides an opportunity to assess the donor's fitness.⁸

The assessment of donor suitability and deferral are excluding donations from those at risk of RTTI, especially those that may have occurred recently and may not be detected by routine screening tests or for which no practical blood screening tests are available.¹¹

The WHO has given recommendations for donor assessment and acceptance and deferral of donors based on selection criteria.⁸ These include lower and upper age limits, donor appearance and inspection, minor illnesses, weight, gender for pregnancy, lactation, breastfeeding, decreasing transfusion-associated acute lung injury (TRALI) risk, vital signs, donor iron status to be determined by hemoglobin (Hb) screening, frequency of donation and iron supplementation, occupation and leisure activities and special considerations for apheresis donations.⁸

The Association for the Advancement of Blood and Biotherapies (AABB) published an Individual Risk Assessment (IRA) Donor History Questionnaire Example Model in 2022 using Canada's eligibility criteria to help the Food and Drug Administration (FDA) and the transfusion community visualize the implementation of this system. The FDA approved an updated questionnaire including IRA in June 2023.^{12,13} The US FDA issued a final guidance document, "Recommendations for Evaluating Donor Eligibility Using Individual Risk-Based Questions to Reduce the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Product", in May 2023 in which FDA's recommendations for donor deferral and re-entry are summarized.¹⁴

Blood donation is one of the most highly controlled practices globally. Safeguarding the safety and efficacy of blood components is critical, as blood is an irreplaceable part of modern clinical therapies.¹¹ The World Health Organization (WHO) and AABB have defined donor health questionnaires. Norway changed its guidelines and procedures in 2019 to include new donors at the Oslo Blood Center. With this change, only anaphylactic reactions provide a reason for deferral, while people with other allergic symptoms can donate. Similarly, those with β -blockers and antihypertensive medications could not donate, while those taking other antihypertensive medications in the revised guidelines remain eligible.¹⁰ The upper age limit has been removed, allowing some healthy elderly individuals to continue to donate. The national guidelines of Norway were revised to defer persons taking permanently or who had been taking injectable drugs. Cannabis use and oral/nasal intake more than one year prior are currently acceptable.¹⁰ Another welcome change is the shorter deferral of 4 months rather than 12 months of people traveling to malaria-endemic countries and donors from South/Latin America.

In Korea, the Blood Management Act was enacted in 1970, and the national blood service was consigned to the Korean Red Cross (KRC) in 1981.¹⁵ In 1998, a 100% voluntary blood donor system was achieved.¹⁵ Donations can be accessed anywhere in the country via the Electronic Health History Assessment System on the KRC website (<https://bloodinfo.net>).¹⁵ Potential donors, who were deferred in the past, are added to this system and deemed ineligible nationally.¹⁶

Uganda is an East African low-income country (LIC), with nearly 20% of its population below the poverty line.¹⁷ Although it has a national blood transfusion service, it needs more blood available for patients. In a study by Checkley et al on "Assessment of Blood Donation and Transfusion", the authors found that > 50% of participants were unaware of the minimum age and weight requirements for blood donation and the greatest blood collection volume.¹⁷ There was an incorrect knowledge of eligibility criteria regarding pregnancy, menstruation, breastfeeding women, and diabetics. Education about donation through informational messaging may, it is hoped, will increase the safe blood supply.¹⁷

In India, blood transfusion services are governed under the Drugs and Cosmetics Rules, initiated in 1945 and amended periodically. In 2011, the upper age limit was increased from 60 years to 65 years. Through another amendment in the act in 2020, donor selection criteria were revised in which after whole blood donation, a plateletpheresis donor shall not be accepted before 28 days.¹⁸

Donor Qualification by Focused Physical Examination

The general health of a donor is evaluated before accepting an individual for donation, and this process is commonly referred to as a focused or mini-physical examination. The broad criteria for donor qualification include vital parameters like temperature, pulse, blood pressure, and Hb/hematocrit (Hct) levels.¹⁹ These criteria are essential to ensure the quality of the blood components and also the donor's safety.¹⁹ There are additional criteria for the apheresis donors, which must meet the regulatory requirements. The AABB Standards for Blood Banks and Transfusion Services (BB/TS) and WHO guidelines for donor examination are two of the most widely followed documents for these criteria.^{8,19} These guidelines define the specific physical requirements and the level of medical supervision required for the assessment. For example, the WHO guidelines state that first-time donors above 60 years of age and repeat donors above 65 years may be accepted if found fit on examination.⁸ The AABB BB/TS also addresses these issues similarly; however, it does not require additional evaluation for older donors. The commonly used vital parameters and their acceptance criteria across different geographical locations are discussed here.²⁰

Pulse

The criteria for measuring pulse before blood donation varies significantly from one country to another. As per the AABB BB/TS and the US FDA regulations, the donor's pulse should be regular and, in the range, of 50–100 beats per minute.¹⁹ Suppose the pulse is irregular or outside these values. In that case, the designated physician must determine if the donor may be accepted after validating that donating would not adversely affect the donor's health. The WHO guidelines have mentioned that a regular pulse in the range of 60–100 beats per minute indicates good health but suggested assessing its role in blood donation.⁹ The National Health Service (NHS, United Kingdom) blood program states that checking the pulse of the blood donor is a good practice. Still, it should not be considered an absolute criterion for donor qualification.

Blood Pressure

The AABB BB/TS and the US FDA regulations (21 Code of Federal Regulations (CFR) 630.10(f)(2)) state that the systolic blood pressure (BP) should be in the range of 90–180 mm of mercury (mm Hg) and diastolic in 50–100 mm Hg.¹⁰ A donor with BP outside these values may be accepted after evaluation by the responsible physician along similar lines for a pulse, as stated earlier. Studies done on this topic, including a systematic literature review, could not establish any association between raised systolic blood pressure and adverse donor reactions.^{20,21} Given these experiences, some blood centers, no longer defer blood donors for BP (either high or low).¹¹

Weight

The weight norms for that population should be considered for establishing the lower weight limit for donors. In the US, 50 kg (110 lbs.) has been set as the lower limit for collecting 500 mL of whole blood. In most other countries, the donor must weigh at least 45 kg to donate 350 mL of blood and at least 50 kg to donate 450 mL of blood. For double red blood cell (RBC) apheresis donations, the donor's weight should be more than 70 kg.⁸

Local Examination and General Appearance

The focused examination also includes an inspection of the antecubital area of the donor for scars or other skin lesions. Common and mild skin disorders are usually not considered a cause for the deferral of the donor. Still, if there is evidence of superimposed bacterial infection, the donor may be deferred. Also, if the lesion interferes with the skin disinfection of the phlebotomy area or there is evidence of intravenous drug use, the donor may be deferred. The general condition of the donor should be observed to rule out malnutrition and the influence of alcohol/intoxication. The donor should not have a fever, breathing difficulties, or persistent cough.

Hemoglobin/Hematocrit Measurement

Hb or Hct measurement before the donation is essential to ensure that blood is not collected from a donor already anemic and that the RBC has an adequate Hb content and Hct to be effective in the recipient. It is also a required parameter for collecting blood using automated platforms. The method for estimating Hb/Hct should be rapid, easy to use, and cause minimum discomfort to the donor. The US FDA has set a lower acceptable limit for Hb at 12.5 g/dL for females and 13.0 g/dL for males.¹⁹ Acceptable ranges vary amongst other countries from 12.5–13.5 g/dL, while some countries have kept a single cut-off level for all donors. Non-invasive methods of Hb measurement have recently been approved in some countries, which do not involve a blood sample but estimate Hb levels through the skin. Hb is measured by following optical changes after a brief blood flow occlusion in a finger (occlusion spectroscopy).

As evident from the above discussion, the selection criteria for voluntary donors have undergone a significant change, especially in the last decade. In many countries, blood collection centers follow the guidelines set by their regional or national regulatory agencies, and these guidelines often change with time as new evidence emerges. These changes aim to maintain the blood supply and are often aligned with demographical factors. For example, relatively less stringent criteria for blood pressure and age will enable more donors in regions with an aging

population to donate blood. A more stringent deferral criteria in the past for certain groups, including men who had sex with men (MSM), had limited the available donor pool. The deferral criteria were changed to 3 months from 1 year by the US FDA in 2020 to expand the donor pool. The 2023 policy guidance by the US FDA has advocated an IDA approach along the lines adopted by various countries and a step towards a more inclusive blood supply.²²

Blood-Center-Defined Donor Eligibility Criteria Cancer

With the advent of better diagnostic and therapeutic options available to patients suffering from malignancy, these patients often present as prospective donors with a history of cured or “in-remission” malignancy. The deferral period for such donors depends upon the type of cancer and the treatment history. WHO and the American Red Cross (ARC) permanently defer individuals with current or cured hematological malignancies from donating blood.^{8,23} These include patients with a history of leukemia or lymphoma, including conditions such as Hodgkin lymphomas, polycythemia rubra vera, essential thrombocythemia, and paroxysmal nocturnal hemoglobinuria.

There is wide variation in accepting recovered patients as blood donors regarding solid tumors. While the ARC takes specific donors who have no cancer for a period, there are certain speculations regarding theoretical risks of transfusion-transmission of tumor cells or oncogenic viruses, which have warranted permanent deferral in some blood transfusion services.^{24–26}

The WHO recommends accepting recovered solid malignant tumors after five years of successful curative treatment and accepting “in-situ” malignant diseases like basal cell carcinoma or cervical carcinoma in situ.⁸ However, as per Indian Blood Transfusion Services, there is a blanket permanent deferral for any malignant condition.

Bleeding Conditions or Blood Diseases

The WHO permanently defers individuals with coagulation factor deficiencies (inherited or acquired).⁸ The Indian Blood Transfusion Services permanently defer all with a history of bleeding disorders and unexplained bleeding tendencies. Hemoglobinopathies such as thalassemia major and sickle cell disease are permanently deferred, while the thalassemia trait can be accepted if the donor meets the minimum Hb threshold for blood donation.^{8,26} Those with sickle cell trait are allowed for whole blood donation by WHO; however, with certain limitations on its use, including to neonates and recipients with sickle cell disease. Red cell enzyme deficiencies with a known history of hemolysis, including the most commonly seen glucose-6-phosphate dehydrogenase (G6PD) deficiency, are permanently deferred.

Heart and Lung Conditions

According to the WHO, cardiovascular disease requires written permission from a cardiologist or physician before donating.⁸ In addition to symptomatic ischemic heart disease, a history of myocardial infarction, symptomatic peripheral vascular disease, and rheumatic fever with evidence of chronic heart disease, several cardiac conditions can lead to permanent blood donation deferral. When assessing the eligibility of individuals with heart disease, consideration should be given to how the disease impacts their ability to tolerate hemodynamic changes caused by blood donation.⁸

The WHO permanently defers donations from donors with severe obstructive airway disease, including those on long-term oral steroid therapy, chronic or recurrent respiratory infections, and breathlessness at rest or with minimal exertion.⁸ Asthmatic patients who are asymptomatic and receiving a nonsteroidal and inhaled steroid maintenance dose may be eligible for blood donation. However, there are differences regarding deferral for asthma patients taking oral or injectable steroids. WHO provides deferral for 14 days after recovery or the last dose; the ARC does not mention deferral for such cases.⁸ The Indian Blood Transfusion Service permanently excludes such patients from blood donation.

Tuberculosis patients are accepted two years after confirmation of cure, while the contacts of tuberculosis individuals are deferred until they are confirmed clear of infection. When assessing those with respiratory disease, the health status of the donor and the risk of transfusion-transmission to the recipient must be considered.⁸

Medications

Usually, most over-the-counter drugs are acceptable for blood donation. For aspirin, there is a deferral period ranging from 72 hours to 5 days (if the blood is used for making platelets). Certain teratogenic medications such as etretinate, acitretin, isotretinoin, and fetotoxic drugs that include dutasteride and finasteride warrant deferral for a specific period (Table 2). It should be noted that not all medication deferrals are evidence-based, and some are based upon the precautionary principle.

Recipient-Specific Designated or Directed Blood Donation Exceptional Medical Need

Sometimes a recipient may benefit from a specific donor.¹¹ The recipient may have multiple antibodies or antibodies to high-prevalence antigens.¹¹ Multiple short-interval donations by a designated individual with a medical need require that the blood collection facility have a policy that requires a request from the patient's doctor and authorization by the blood center's medical director.¹¹ The donor must meet all other allogeneic donor requirements with the expectation of frequency. If an urgent need arises, blood can be released before RTTIs results are complete but must be labeled and managed per the CFR.^{11,28}

Directed Blood Donations

Patients can request the blood center collect directed donors, usually family members and friends. While directed donations have declined in recent years, this practice remains and is often driven by an inaccurate perception of the risk of RTTIs.²⁹ There is no evidence of improved safety. These donors may feel coerced to donate, which could reduce blood safety. The confidentiality of positive test results may be challenging.¹¹

Directed donors must meet all criteria as allogeneic donors, and their blood can be transfused to others if not used by the intended recipient.¹⁹ The blood center should convey its directed donation procedures so expectations of unit availability are clear to the hospital, ordering physician, and patient. This should include the mandated interval between collection and availability to the patient. The hospital should have a defined policy for the release of directed donations to other patients should the unit not be used.¹¹

Autologous Blood Donations

In the 1980s, when the awareness that allogeneic transfusions are associated with several adverse effects, such as the transmission of RTTIs and hemolytic transfusion reactions, autologous blood transfusions (ABT) gained popularity since it was safer and offered the best compatibility for the patient.³⁰

Pre-procedure autologous blood donation, perioperative hemodilution, perioperative salvaging of blood, and recombinant erythropoiesis-stimulating agents (ESA) therapy and combinations of these approaches come under the purview of autologous donations.³¹ ABT is not a recent concept; blood shed during surgery was reinfused as early as 1818, and preoperative donation of autologous blood was promoted since the beginning of blood banking in the 1930s.³²

Table 2 A Selection of Medications and Deferral Periods for Blood Donors

Medication	Deferral Period (Provided as a Range from Minimum to Maximum Across Various Guidelines) ^{23,26,27}
Aspirin	2 to 5 days
Finasteride	28 days to 1 month
Etretinate, Acitretin or Isotretinoin	28 days to 3 years
Antibiotics for acute infection	14 days after completion

Less stringent criteria are used for autologous donation selection compared to allogeneic donation. The AABB BB/TS mandates no age or weight restrictions, and the minimum Hb level for the donor-patient should be 11.0 g/dL and hematocrit 33%.¹⁹ Donations may be made more than once per week.^{11,19}

Autologous blood, however, is not necessarily 100% safe. Transfusion errors, transfusion reactions, adverse reactions during blood donation, bacterial contamination during processing and storage, and incompatible transfusions due to clerical errors continue to occur.³³ In a hospital-based study, autologous blood units were implicated in 2.1% of all adverse transfusion reactions.³⁴ Furthermore, autologous transfusions are logistical and resource-intensive.

A successful ABT requires an organized commitment between the surgical and blood center teams to maximize the donated blood's utilization. Blood loss must be anticipated individually to determine the number of blood units to be collected, and the surgery schedule must be decided early enough to avoid the outdating of collected units.³⁵ The cost per unit of autologous blood tends to be significantly higher than that for allogeneic blood units.³⁶ Data reveals that patients who had autologous blood deposited tend to be transfused at higher hematocrits than patients receiving allogeneic transfusions.³⁷ More than half of all units collected are discarded.

Strategies for making autologous transfusions cost-effective have been proposed, which include developing a "maximum surgical blood order schedule" or MSBOS for autologous blood, similar to allogeneic units, not screening autologous blood units for RTTIs, using whole blood instead of processing into components, and requiring patients to pay for the additional costs of collecting and storage.^{38,39} However, the cost-benefit of these actions still needs to be investigated and proven.

Perioperative hemodilution, which includes acute normovolemic hemodilution, is a type of autologous donation where the autologous blood unit is collected either just before or soon after induction of anesthesia, and intravenous crystalloid or colloid solution is used to sustain the blood volume. Since perioperative hemodilution does not involve processing, it may be more cost-effective and feasible as one constituent of a multifaceted approach to bringing down blood use.⁴⁰

In a survey conducted at the Cleveland Clinic among patients undergoing ABT, only 20% cited fear of infection, and 68% had opted for an autologous donation because their physician had recommended it.⁴¹ Even though an autologous donation is recognized as a part of PBM, perioperative strategies to minimize blood loss, based on two Cochrane reviews of good quality, it is not recommended for routine use because the risk of receiving RBC transfusion (allogeneic and autologous) increases due to lowered Hb levels.^{42,43} Autologous blood donation is recommended only in certain situations, such as a patient with a rare blood group when transfusion requirements cannot be met with allogeneic blood.

Significant developments in window period reduction, enhanced sensitivity of the screening assays, and pathogen reduction technology (PRT) have improved blood safety considerably. This has led to a decline in ABT. In many parts of the world, particularly in high-income countries, autologous donations have all but disappeared. Autologous donation can result in potential harm to the patient, who can develop iron deficiency with or without anemia).

Replacement Donors

In high-income countries (HIC) with well-established blood transfusion services, the demand for blood and blood products is met entirely from blood donated voluntarily, while in low and middle-income countries (LMIC), blood shortages are common and chronic.⁴⁴ Families and friends often provide blood for patients requiring transfusion or paid donation supplements inventory in countries with low rates of voluntary blood donation.⁴⁵

A replacement blood donation occurs mainly when the hospital staff asks the patient's relatives to provide a specified number of blood donors for every patient who requires a transfusion or undergoes surgery.⁴⁶ Patients may also prefer blood from family members or friends rather than "strangers" because they supposedly "know" their family members or friends (FRD).⁴⁶ Their keenness to donate blood either to save the life of a loved one or their fear of distressing or displeasing family may cause them to withhold information about their health status or lifestyle behavior.⁴⁶ Studies have also shown higher rates of discard in the blood units from replacement donors due to the presence of RTTI markers.^{47–50} Another impact of asking for blood on a replacement basis is on its subsequent use. Physicians are pressured by the patient and their family members for irrational transfusions, regardless of patient need and risks associated with transfusion. This widens the gap between supply and demand and deprives blood and blood products for patients who

require it. The issue of blood safety was addressed at the first World Health Assembly resolution (WHA28.72) a half-century prior.⁵¹

The WHO Global Database on Blood Safety has identified several challenges that need to be addressed to promote voluntary blood donation,⁴⁵ including commitment and support of the government towards infrastructure, human and financial resources, acknowledgment of transfusion as an essential part of the health care, development, and enactment of a national blood policy and a plan with the distribution of supplies are all crucial to achieving and sustaining a 100% voluntary blood donation program.⁴⁴ Public trust in the blood center's ability to support transfusion needs can be earned only over an extended amount of time. Still, it can be lost very swiftly, adversely affecting the trustworthiness and support of donors, partner organizations, and the public.⁴⁴ In short, sustaining a 100% voluntary blood donation program is financially and resource-intensive.

Recent studies conducted in four sub-Saharan African countries indicate FRDs and first-time voluntary blood donors (VBD) were epidemiologically undistinguishable regarding the RTTI marker status.^{52–55} The prevalence of RTTI markers was significantly lower only in VBDs who donated within a 12-month interval.⁵²

The hospital-based FRD system has been in existence for many years. It is providing a maintainable blood inventory, especially in communities and countries without access to a centralized, voluntary donor system.⁵⁶ Instead of completely neglecting FRDs, the safety of the donated units from FRDs can be enhanced by creating awareness about safe blood and enhanced pre-donation counseling, including the option for self-deferral, as well as adherence to donor selection criteria and employing sensitive screening assays for RTTIs. FRDs can be considered a viable, sustainable, and cost-effective source of blood for transfusion as a bridge until a committed voluntary blood donation program is established.

Aging Populations (US)

The number of transplants performed in Europe and associated countries continues to rise, with 47,468 hematopoietic stem cell transplants in 42,901 patients [19,630 allogeneic (41%) and 27,838 autologous (59%)] reported by 701 centers in 50 countries in 2018.^{57–59}

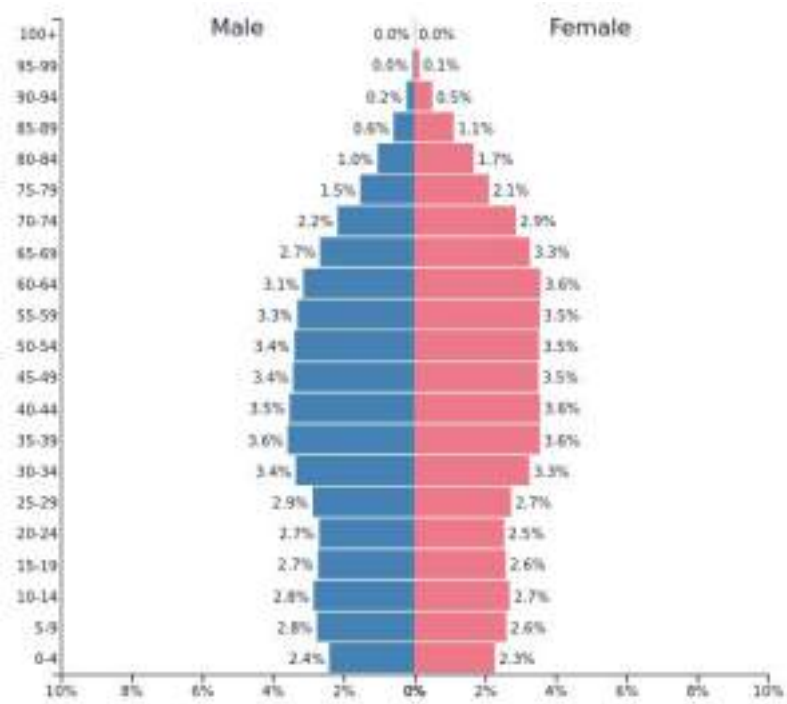
Blood transfusion is a transfer of blood from donors to recipients. These two groups differ considerably in their demographic structure.⁵⁸ Donors are an essential “powering” element for blood services.⁵⁸ The donor population is young and healthy, while most patients requiring blood are of an older demographic.⁵⁸ These significant age differences lead to demographic trends important for future blood supply.⁵⁸

Globally, the population is shifting to an older demographic due to increased life expectancy and the recent decrease in birth rates with stabilization at a current low level.⁶⁰ Adequate blood supply of a population requires the right number of blood donations and the demand for blood transfusions. Because most blood donors are 18 (or 16 years in some countries) to 65 years, and most blood recipients are >65 years, the ratio between these two population groups is relevant for the blood supply and demand relationship.⁵⁹ This the number of recipients will change substantially in all European and North American countries.

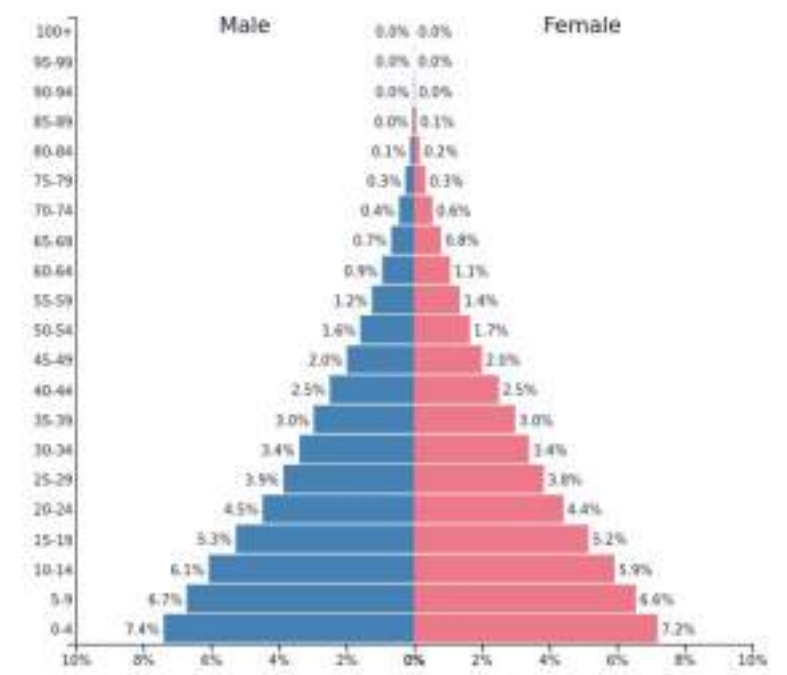
It is evident from the population pyramids of 2023 of various continents (Figure 1) that the demography of North America, Europe, and the United Kingdom (UK) is changing towards an aging population; which means the donor pool is shrinking with an increase in the recipient population while in population pyramid of Africa, the base is broad indicating predominance of the younger, pediatric population which are yet to enter donor pool with lesser of the elderly population. Asia and particularly India has an expansive donor pool with a less elderly recipient population. These different demographics in different continents will affect the blood supply, more in western countries, in the next ten years.

In the last few decades, more studies on PRT have been done, but changing the demography of HIC poses a much higher risk for the safety of blood supply than PRT which is currently available for plasma and platelets in some countries and under investigation for RBCs in others. In 1994, Vamvakas & Taswell forecasted future US blood requirements to increase by 64% from 1989 until 2030, while blood collection would increase by only 12%, suggesting a shortfall of nearly 4 million RBC units by 2030 in US.⁶¹

A study by Zou et al for the ARC found that repeat donations by donors over the age of 50 increased from 22.1% to 34.5% from 1996 through 2005, while repeat donations by donors of less than 50 years age group decreased from 59% to 48% during the same period.⁶² They predicted a severe shortage of blood components unless measures are taken to



Europe



Africa

Figure 1 It is evident from population pyramids of 2023 that the demography of Europe is changing towards an aging population, which means the donor pool is shrinking with an increase in the recipient population while in the population pyramid of Africa, the base is broad indicating a predominance of the younger, pediatric population which are yet to enter donor pool with lesser of the elderly population. These different demographics will affect the blood supply in the next ten years. Reprinted from Population Pyramids of the World from 1950 to 2100. Available from: <https://www.populationpyramid.net/world/2023/>.

increase the inventory or reduce the use considerably. The European blood donor population data tends to be sparser, possibly related to underreporting studies or language issues.⁶²

Currie et al predicted a future increase in transfusions for Wales in 2004.⁶³ It would increase over the next quarter century, achieving a 29% increase in demand by 2026 compared with 1999, primarily due to an aging population with higher transfusion rates.⁶³ Requests for RBCs are increasing in Taiwan, and authors anticipated that this would endure due to the aging population.⁶⁴ In Finland, there is a marked increase in RBC use beginning at around age 50.⁶⁵ Those patients age 70 and older have an eightfold higher RBC consumption than their counterparts ages 20–40 years.⁶⁵

With pronounced demographic changes occurring globally and different medical practices between countries, it is essential to monitor the donor and recipient age distributions regularly. Some radical actions such as extending the age limit for donations, reversing certain donor deferrals, the impact of changing demography on blood demand and supply, and improving various PBM strategies to prevent blood shortages or wastage due to overproduction will need to be explored.

Infrastructure Concerns (US)

For a successful blood center, achieving 100% voluntary, non-remunerated blood donors (VNRBD) are considered a landmark for the available resources. However, monetary allocations for rewards and the availability of modern infrastructure play a vital role in achieving the above status. Contrarily, blood donor programs are often perceived to be inexpensive to run, and they receive lower priority in funds allocations in governmental-run blood centers and more so in LMICs. This fund insufficiency affects donor recalls, the operation of mobile donor vehicles, and the maintenance of vehicles. While much work has been done globally on blood availability, blood and transfusion safety, and appropriate use of blood and blood components, each country's approach to funding the blood supply and provision of transfusion needs to be standardized and is highly variable. Funding varies among countries, with some countries with no specific mechanism and others using cost recovery.

Within blood centers, gaps are well known, more in LMICs, concerning competent trained staff, which gets compounded in LMICs. There is a need for training and education to ensure adequate blood supplies and transfusion safety, especially in LMICs.

Blood should only be collected in settings with an appropriate number of trained staff to perform the procedures safely. Staff should be trained in communication skills for successful donor information, education, and motivation. Notifications and counseling, especially when adverse events occur, require qualified and trained staff with excellent interpersonal talents and the capability to provide support and care. Human resources are needed to strengthen infrastructure, particularly graphic design and marketing experts, to create effective posters and leaflets, organize traditional and social media campaigns, and hold public talks.

RBC serology and molecular testing are highly variable in many parts of the world. In some countries and regions, pre-transfusion anti-human globulin crossmatch is well established, while tile crossmatch is performed in other places. Extended red-cell phenotyping is performed only in some hospital-based transfusion services, more so in sub-Saharan African countries where there is often a lack of reagents to conduct tests or technical skills.⁶⁶ The situation gets further compounded for multiply-transfused sickle cell patients who have developed multiple alloantibodies. Other infrastructure and resource constraints are faced with procuring sufficient screening for red cell antibodies, RTTIs, hemovigilance, and quality assurance.

Similarly, screening tests for RTTIs with low sensitivity led to the high prevalence of RTTIs. Due to a shortage of money and technical ability, in most hospitals in sub-Saharan Africa, the prevalence of HIV, HBV, HCV, and syphilis in blood donors is 10.9% in Nigeria, 9.4% in Kenya and 6.6% in Ethiopia.⁶⁶ Only a few countries in Africa are using nucleic acid tests (NAT), namely Ghana, Egypt, Namibia, and South Africa.⁶⁶

Using technology, the internet, various blood establishment computer systems, or implementing blood center services based on cloud computing increases efficiency and safety. However, it is a significant infrastructure challenge for resource-constrained countries where basic component production and fractionation is a distant dream. In sub-Saharan African countries, some blood centers have no constant electricity supply. Generators can make electricity supply for blood bank refrigerators. Still, the energy required for deep freezers to store plasma and cryoprecipitate often exceeds their capacity, or freezers break down due to frequent electricity failures, power

outages, and fluctuating voltage. This leads to the thawing of plasma several times within a few weeks, requiring it to be discarded.⁶⁷

Implementation science, a term coined by Custer et al in 2018, evaluates the acceptability, affordability, appropriateness, feasibility, and sustainability of interventions to improve the availability of blood and blood components in LMICs.⁶⁸ Where applicable, every government, federal or state, and private organization needs to allocate adequate budgets to have effective and safe blood donations and transfusions.

Increased Demand

In the US, though around 6.8 million donors attempt to donate, only approximately 10% of that population is eligible.²³ Furthermore, global crises, such as the recent SARS-CoV-2 pandemic, have further hindered blood and blood product collection. It was estimated by the ARC that during the height of the pandemic, there was a 62% drop in blood donations, specifically from school drives, which supply most of their blood products.²³ The pressure for more blood donations has witnessed a significant upsurge in recent years, influenced by various factors. The expanding population and the increasing number of medical procedures requiring blood transfusions are the primary factors behind this surge. As more individuals undergo surgeries and medical treatments, the demand for blood products intensifies. Natural disasters and emergencies also contribute to the elevated demand, as they often result in substantial loss of life and injuries, necessitating a greater supply of blood. The aging population is another contributing factor to the augmented need for blood donations. As individuals age, they become more prone to developing health conditions that require blood transfusions, such as anemia or cancer. Moreover, advancements in medical technology have introduced novel treatments and procedures that rely on utilizing blood products, further propelling the demand.

New donors are constantly needed to meet the increasing demand for blood donations. Blood centers rely on the generosity of volunteer donors to keep their supplies at a level that can meet the needs of patients. Regular blood donations are essential to ensure that a sufficient supply is available when it is needed. Therefore, individuals must consider donating blood regularly to help others in need and meet the growing demand.

Role of Patient Blood Management

Blood is a common therapeutic intervention during hospitalization. More than 15 million blood components (red blood cells, platelets, and plasma) are transfused annually in the US.⁵ Both blood product utilization and collection have decreased over the past decade; however, the decline is now appearing to slow down,⁵⁹ and keeping a steady balance of supply and demand is crucial. Blood product utilization declined primarily due to the advancement of less invasive surgical techniques and revised transfusion guidelines from professional medical societies.

PBM is a comprehensive, evidence-based, multidisciplinary process focused on patients for optimal utilization of blood products and limiting blood transfusion risks. The core principles of PBM are appropriate preoperative anemia management and optimization of RBC mass, limiting blood loss during surgeries, and rational transfusion of blood products.⁶⁹ PBM was primarily aimed at surgical patients; however, its scope has also gradually evolved for medical conditions. Reduced blood product utilization has been shown in several studies with PBM implementation. A retrospective study of 605,046 patients showed a 41% reduction in transfused blood product units (RBC, fresh frozen plasma, platelets) per admission and improved patient outcomes after initiating a health systemwide PBM program.⁷⁰ Another study showed a significant reduction in RBC (39%) and platelet (35%) transfusions per admission in patients on intense chemotherapy for acute leukemia or hematopoietic stem cell transplant.⁷¹

A comprehensive PBM program not only focuses on optimal blood product utilization but also has a component for reducing blood product wastage. Although a fraction of blood product wastage is inevitable, minimizing avoidable wastage can play a crucial role in blood product supply conservation. With an average rate of 1 to 5% blood product wastage, a few hundred thousand blood products are discarded annually in the US. A multidisciplinary approach, including implementing effective inventory management for tracking and storing blood products and communication with ordering providers, can be used to minimize blood product wastage. Heitmiller et al used an interdisciplinary team and the Lean Six Sigma methodology. They reduced overall RBC wastage by approximately 50% (4.4% to less than 2%).⁷² A recent study by Levin et al showed that even a low-cost initiative such as physician-to-physician

communication could significantly reduce blood product wastage.⁷³ In a dwindling donor supply setting, there is a greater need to promote PBM as a standard of care.

Promote Understanding of the Importance and Culture of Patient Blood Management

According to data from clinical trials and published clinical guidelines, a limited blood transfusion strategy is recommended as the best practice for hospitalized, stabilized patients. A crucial process improvement for lowering variability in transfusion practices and clinical outcomes is creating and implementing PBM programs.

It can be challenging to improve practice, especially when there is little enthusiasm for change. Utilizing practice development teams that have already been established inside the organization and employees who are knowledgeable about the technique should be considered.

It requires collaborative and team effort to practice change and implementation. It may work locally with the help of short-term project teams but needs organizational, structural, and permanent process change to be effective at hospital / institute or multi-hospital levels.

Understanding and utilizing hospitals' unique settings and cultures can help bring about change.

Especially from within the organization groups, leaders are essential. Executive leadership is crucial to support and promote a space for PBM inside the hospital. The most important resources for getting physicians to pay attention to the research and the need for practice change are reputed clinicians in the institute. It is essential to start thinking about clinical champions in the affected groups as soon as possible. Local doctors must be involved in the co-design process to ensure projects' relevance to a specific environment and increase their chances of success. Emphasis should also be made to engage non-clinical stakeholders as altered processes may also affect them. Patients are kept at the center of care when changes are made, a widely accepted principle in PBM and all models of care.

Support from C-Suite

Implementing a PBM program at a hospital/transfusion service requires input and support on many levels. The program depends on a multidisciplinary committee of doctors, nurses, laboratory scientists, and management. It is imperative to have executive support for the successful startup of a program. Starting a PBM program involves many changes that clinicians must abide by, as well as startup costs associated with supporting the program (eg, hospital information systems, education, and staff support).⁷⁴

C-suite support is crucial for the success of PBM programs. The C-suite consists of top-level executives, such as the chief executive officer (CEO), chief operating officer (COO), and chief financial officer (CFO), who have the power to make crucial decisions and allocate resources within an organization. The C-suite can demonstrate its commitment to improving patient care and reducing healthcare costs by supporting PBM programs.

PBM programs aim to optimize the use of blood products to minimize blood transfusions, reduce the risk of transfusion-related adverse events, and improve patient outcomes. C-suite support for these programs can include providing financial resources to implement new protocols and technology and encouraging interdepartmental collaboration and staff education. In addition, C-suite leaders can advocate for the program to stakeholders, including other executives, physicians, and regulatory bodies, to help secure the necessary support and resources.⁷⁴

Having the support of the C-suite is also crucial for the sustainability and growth of PBM programs. The C-suite can ensure that the program remains a priority for the organization, even as other initiatives are introduced. In addition, by providing ongoing support and resources, the C-suite can help ensure that the program successfully improves patient care and reduces healthcare costs over the long term. McKinney mentions an annual blood acquisition reduction of \$1.6 million US dollars from their PBM program and a 10% decrease in cardiac surgery costs per case.⁷⁵ They also provide annual education for providers, and their electronic ordering system allows for real-time alerts. Depending on the contract and current system set up for the ordering clinician, this can require startup capital. Annual education also needs funds for provider wages as well as educational content. Overall, cost savings from successful programs support the initial startup costs.

The Effects of COVID on the Blood Supply

Due to the impacts of COVID-19 on blood donation efforts, for several months in the first half of 2021, a severe national blood shortage plagued the US during the first half of 2021. On June 17, 2020, a joint statement released by AABB and the blood centers of the US urged hospitals to implement PBM strategies. In addition, the WHO issued a policy brief relating to implementing a PBM program. The three pillars of PBM include detecting and managing anemia and iron deficiency, minimizing blood loss and optimizing coagulation, and leveraging and optimizing the patient-specific physiological tolerance of anemia.⁷⁶

A systematic review of the literature during March and April 2020 provided recommendations to consider, namely, the impact COVID-19 has on patient need for transfusion, donor and donation factors impacting supply, PBM implementation, and prioritization of patient transfusion needs.⁷⁷ Lessons learned from the COVID-19 pandemic may assist transfusion facilities in future planning during similar supply shortages. These efforts highlight ways facilities can start or improve upon existing PBM programs.

With the cancellations of elective procedures early on in the COVID-19 pandemic, some facilities saw a concurrent reduction in blood utilization, so blood shortages were often avoided.⁷⁸ Similarly, in one French institution Delabranche et al⁷⁹ provided, a year-to-year retrospective analysis study showed that a reduction of patient influx due to the cancelation of elective surgeries despite an 11% reduction in blood donations, led to a manageable supply of blood available for patients, with no needed transfusions being canceled. Finally, for some facilities with pre-existing PBM programs before the pandemic, whether formalized or in-house implementations,⁸⁰ were less impacted initially by the shortages.

International Society of Blood Transfusion (ISBT) survey researchers conducted a 37-question anonymous survey of transfusion medicine specialists across the US.⁸¹ Highlights of this study showed that 98% of respondents experienced a blood shortage, 35.3% of institutions altered their blood inventory number or composition of products as well as blood suppliers, 78% of respondents reported that blood conservation strategies resulted in reduced blood product usage, and 38.1% reported decreased blood product wastage.

The authors of one US proof of concept study concluded that an ultra-restrictive transfusion approach for non-bleeding, non-COVID, or non-cardiac ischemic patients (< 6 g/dL Hb threshold) led to adequate blood inventory management during the pandemic.⁸²

As a result of shortages experienced during COVID-19, some hospitals modified existing massive transfusion protocols (MTPs). For example, one author's (CH) institution modified quantities of each round while maintaining expected product ratios. Instead of four units of RBCs and plasma sent in each round, two units of each product were sent. After the pandemic, a decision was made to keep the reduced quantities as part of each MTP round, with better inventory management and reduction of wastage as factors to support this change. The National Transfusion Committee in the UK further describes a triage tool for patients requiring massive transfusion to ensure that the most significant number of life years can be saved ethically and equitably.⁸³

PBM also has a role in emergency medicine. Beverina et al, describe how a hospital embraced PBM and broke with the tradition of transfusion therapy for treating severe iron deficiency anemia (IDA). In 2018 the Emergency Department and anemia clinic (AC) adopted a synchronized approach to treating patients with severe IDA with the objective of offering, as swiftly as possible, the most appropriate treatment at the first point of admission and subsequently proceeding with additional therapy and follow-up.⁸⁴

Conclusion

This manuscript has shown that blood donation and PBM go hand in hand. With the many challenges blood centers face, there must be an adequate number of donors for the blood products needed. At the time of writing of this manuscript, blood can only be obtained from donors and not manufactured. As the population continues to age in certain regions, those who were previously donors may need to leave the pool and potentially become blood product recipients. In an effort to maximize patient safety, however, transfusion medicine practice culture needs to shift towards a PBM approach, with hospitals implementing it as an important tool to minimize the risks of allogeneic blood transfusion and optimize the

precious gift of blood donation.⁸⁵ In summary, a robust blood donor pool and good stewardship of the blood supply through PBM ensure evidence-based transfusions, and product wastage is minimized for a safe and adequate blood supply as we move into the second quarter of the 21 century.

Disclosure

All authors report no conflicts of interest in this work.

References

1. US Department of Health and Human Services Report to Congress on the Adequacy of the National Blood Supply 2020; 2020. Available from: https://www.aabb.org/docs/default-source/default-document-library/positions/hhs-report-to-congress-on-The-adequacy-of-The-national-blood-supply.pdf?sfvrsn=aeca20ff_4. Accessed December 16, 2022.
2. Mulcahy A, Kapinos K, Briscoe B, et al. *Toward a Sustainable Blood Supply in the United States: An Analysis of the Current System and Alternatives for the Future*. Rand Corporation; 2016.
3. Cohn CS, Pagano MB, Allen ES, et al. How do I manage long-term blood component shortages in a hospital transfusion service? *Transfusion*. 2020;trf.15857. doi:10.1111/trf.15857
4. McCarthy LJ. How do I manage a blood shortage in a transfusion service? *Transfusion*. 2007;47(5):760–762. doi:10.1111/j.1537-2995.2007.01187.x
5. Mowla SJ, Sapiano MRP, Jones JM, Berger JJ, Basavaraju SV. Supplemental findings of the 2019 National Blood Collection and Utilization Survey. *Transfusion*. 2021;61(S2):S11–S35. doi:10.1111/trf.16606
6. Shander A, Hardy JF, Ozawa S, et al. A Global definition of patient blood management. *Anesth Analg*. 2022;135(3):476–488. doi:10.1213/ANE.0000000000005873
7. Blanton K. What's in a name: the role and impact of the Transfusion Safety Officer. In: *AABB Annual Meeting Highlights Conference & Exposition in the Middle East*; 2020.
8. World Health Organization. *Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation*. World Health Organization; 2012.
9. World Health Organization. The urgent need to implement patient blood management: policy brief; 2021. Available from: <https://apps.who.int/iris/bitstream/handle/10665/346655/9789240035744-eng.pdf>. Accessed November 7, 2023.
10. Nissen-Meyer LSH, Seghatchian J. Donor health assessment – when is blood donation safe? *Transfus Apher Sci*. 2019;58(1):113–116. doi:10.1016/j.transci.2018.12.016
11. Kessler D, Rossmann S. Allogeneic and autologous blood donor selection. In: Cohn C, Delaney M, Johnson S, Katz L, editors. *Technical Manual*. 20th ed. AABB Press; 2020.
12. Association for the Advancement of Blood and Biotherapies. Individual risk assessment donor history questionnaire example model using Canada's eligibility criteria; 2023. Available from: <https://www.aabb.org/docs/default-source/default-document-library/resources/individual-risk-assessment-dhq-example-model-using-canadas-eligibility-criteria.pdf>. Accessed May 19, 2023.
13. US Food and Drug Administration. *Implementation of Acceptable FullLength and Abbreviated Donor History Questionnaires and Accompanying Materials for Use in Screening Donors of Source Plasma*; 2023.
14. US Food and Drug Administration. *Recommendations for Evaluating Donor Eligibility Using Individual Risk-Based Questions to Reduce the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products*. US Food and Drug Administration; 2023.
15. Cho JE. National blood management system and the direction of government policy in Korea. *Korean J Hematol*. 2010;45(2):81. doi:10.5045/kjh.2010.45.2.81
16. Kim HO. Current state of blood management services in Korea. *Ann Lab Med*. 2022;42(3):306–313. doi:10.3343/alm.2022.42.3.306
17. Checkley L, Motwani G, Wange IC, et al. Assessment of blood donation and transfusion in Eastern Uganda: a Mixed-Methods Study. *Ann Glob Health*. 2019;85(1). doi:10.5334/aogh.2426
18. Ministry of Health and Family Welfare. Drugs and Cosmetics Act, 1945. Final G.S.R. 166(E) _Amendment in Part X B & Part XII B pertains to Blood centre and Blood components. The Gazette of India: Extraordinary; 1945. Available from: https://cdsco.gov.in/opencms/opencms/system/modules/CDSCO.WEB/elements/download_file_division.jsp?num_id=NTc2MQ. Accessed January 21, 2023.
19. Gammon RR, Boyd T, Chanez T. *Standards for Blood Banks and Transfusion Services*. 33rd ed. Bethesda, MD: AABB; 2022.
20. Roubinian NH, Plimier C, Woo JP, et al. Effect of donor, component, and recipient characteristics on hemoglobin increments following red blood cell transfusion. *Blood J Am Soci Hematol*. 2019;134(13):1003–1013.
21. De Kort W, Mayr W, Jungbauer C, et al. Blood donor selection in European Union directives: room for improvement. *Blood Transfus*. 2016;14(2):101. doi:10.2450/2015.0148-15
22. Miller AS, Cahill S, Mayer KH. FDA's 2023 Policy Update—Promoting Safety and Inclusivity in Blood Donation. *JAMA Health Forum*. 2023;4(8):e232388. doi:10.1001/jamahealthforum.2023.2388
23. Blood Donor Eligibility Criteria. Red Cross blood services; 2023. Available from: <https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements/eligibility-criteria-alphabetical.html>. Accessed March 19, 2023.
24. Memon A, Doll SR. A search for unknown blood-borne oncogenic viruses. *Int J Cancer*. 1994;58(3):366–368. doi:10.1002/ijc.2910580310
25. Dzik WH, Okayama A. Can blood transfusion transmit disease-producing genes? *Transfusion*. 1999;39(8):795–800. doi:10.1046/j.1537-2995.1999.39080795.x
26. NACO, NBTC. Guidel. Blood Donor Sel; 2017. Available from: <http://nbtc.naco.gov.in/page/publications/>. Accessed March 19, 2023.
27. Blood donor selection guideline on assessing donor suitability for blood donation; 2012. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK138219/>. Accessed December 24, 2022.
28. Government Publishing Office. *21 CFR 600-799. Code of Federal Regulations*. Government Publishing Office; 2022.
29. Dorsey KA, Moritz ED, Steele WR, Eder AF, Stramer SL. A comparison of human immunodeficiency virus, hepatitis C virus, hepatitis B virus, and human T-lymphotropic virus marker rates for directed versus volunteer blood donations to the American Red Cross during 2005 to 2010. *Transfusion*. 2013;53(6):1250–1256. doi:10.1111/j.1537-2995.2012.03904.x

30. Etchason J, Petz L, Keeler E, et al. The cost effectiveness of preoperative autologous blood donations. *N Engl J Med*. 1995;332(11):719–724. doi:10.1056/NEJM199503163321106
31. Domen RE. Preoperative autologous blood donation: clinical, economic, and ethical issues. *Cleve Clin J Med*. 1996;63(5):295–300. doi:10.3949/ccjm.63.5.295
32. Goodnough LT. Autologous Blood Donation. *Anesthesiol Clin North Am*. 2005;23(2):263–270. doi:10.1016/j.atc.2004.07.003
33. Yomtovian R. Practical aspects of preoperative autologous transfusion. *Am J Clin Pathol*. 1997;107(4 Suppl 1):S28–35.
34. Domen R. Adverse reactions associated with autologous blood transfusion: evaluation and incidence at a large academic hospital. *Transfusion*. 1998;38(3):296–300. doi:10.1046/j.1537-2995.1998.38398222875.x
35. Letts M, Perng R, Luke B, Jarvis J, Lawton L, Hoey S. An analysis of a preoperative pediatric autologous blood donation program. *Can J Surg*. 2000;43(2):125–129.
36. Rock G, Berger R, Filion D, et al. Autologous blood programs: what is happening? *Transfus Apher Sci*. 2008;39(3):193–197. doi:10.1016/j.transci.2008.09.014
37. Axelrod F, Pepkowitz S, Goldfinger D. Establishment of a schedule of optimal preoperative collection of autologous blood. *Transfusion*. 1989;29(8):677–680. doi:10.1046/j.1537-2995.1989.29890020438.x
38. Birkmeyer JD, Goodnough LT, AuBuchon JP, Noordsij PG, Littenberg B. The cost-effectiveness of preoperative autologous blood donation for total Hip and knee replacement. *Transfusion*. 1993;33(7):544–551. doi:10.1046/j.1537-2995.1993.33793325048.x
39. Birkmeyer JD, AuBuchon JP, Littenberg B, et al. Cost-effectiveness of preoperative autologous donation in coronary artery bypass grafting. *Ann Thorac Surg*. 1994;57(1):161–168. doi:10.1016/0003-4975(94)90386-7
40. Stehling L, Zauder H. Controversies in transfusion medicine. Perioperative hemodilution: pro. *Transfusion*. 1994;34(3):265–268. doi:10.1046/j.1537-2995.1994.34394196628.x
41. Domen RE, Ribicki LA, Hoeltge GA. An analysis of autologous blood donor motivational factors. *Vox Sang*. 1995;69(2):110–113. doi:10.1111/j.1423-0410.1995.tb01679.x
42. Henry DA, Carless PA, Moxey AJ, O'Connell D, Ker K, Fergusson DA. Pre-operative autologous donation for minimising perioperative allogeneic blood transfusion. *Cochrane Database Syst Rev*. 2001;2010(4). doi:10.1002/14651858.CD003602
43. Henry DA, Moxey AJ, Carless PA, et al. Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. In: editor, Henry DA. *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd; 2001. doi:10.1002/14651858.CD001886.pub2
44. World Health Organization. Towards 100% voluntary blood donation: a global framework for action; 2010. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK305666>. Accessed January 21, 2023.
45. National AIDS Control Organisation. Ministry of Health and Family Welfare. Government of India. Voluntary blood donation programme - an operational guideline; 2007.
46. Ad-Drees A. Attitude, belief and knowledge about blood donation and transfusion in Saudi population. *Pak J Med Sci*. 2008;24(1):74–79.
47. Matee MI, Magesa PM, Lyamuya EF. Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis infections among blood donors at the Muhimbili National Hospital in Dar es Salaam, Tanzania. *BMC Public Health*. 2006;6(1):21. doi:10.1186/1471-2458-6-21
48. Sharma RR, Cheema R, Vajpayee M, et al. Prevalence of markers of transfusion transmissible diseases in voluntary and replacement blood donors. *Natl Med J India*. 2004;17(1):19–21.
49. Sultan F, Mehmood T, Mahmood MT. Infectious pathogens in volunteer and replacement blood donors in Pakistan: a ten-year experience. *Int J Infect Dis*. 2007;11(5):407–412. doi:10.1016/j.ijid.2006.10.004
50. Mremi A, Yahaya JJ, Nyindo M, Molle E. Transfusion-transmitted infections and associated risk factors at the Northern Zone Blood Transfusion Center in Tanzania: a study of blood donors between 2017 and 2019. *PLoS One*. 2021;16(3):e0249061. doi:10.1371/journal.pone.0249061
51. Twenty-Eighth World Health Assembly. *WHA28.72-Utilization and Supply of Human Blood and Blood Products*. Geneva: Twenty-Eighth World Health Assembly; 1975.
52. Asenso-Mensah K, Achina G, Appiah R, Owusu-Ofori S, Allain JP. Can family or replacement blood donors become regular volunteer donors? *Transfusion*. 2014;54(3pt2):797–804. doi:10.1111/trf.12216
53. Allain JP, Sarkodie F, Asenso-Mensah K, Owusu-Ofori S. Relative safety of first-time volunteer and replacement donors in West Africa. *Transfusion*. 2010;50(2):340–343. doi:10.1111/j.1537-2995.2009.02444.x
54. Loua A, Nze Nkoure G. Relative safety of first-time volunteer and replacement donors in Guinea. *Transfusion*. 2010;50(8):1850–1851; author reply 1851–2. doi:10.1111/j.1537-2995.2010.02718.x
55. Mbanya DN, Feunou F, Tayou TC. Volunteer or family/replacement donations: are the tides changing? *Transfusion*. 2010;50(8):1849–1850. doi:10.1111/j.1537-2995.2010.02656.x
56. Bates I, Hassall O. Should we neglect or nurture replacement blood donors in sub-Saharan Africa? *Biologicals*. 2010;38(1):65–67. doi:10.1016/j.biologicals.2009.10.013
57. Passweg JR, Baldomero H, Chabannon C, et al. The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-T's come into focus. *Bone Marrow Transplant*. 2020;55(8):1604–1613. doi:10.1038/s41409-020-0826-4
58. Greinacher A, Fendrich K, Hoffmann W. Demographic changes: the impact for safe blood supply. *Transfus Med Hemother*. 2010;37(3):141–148. doi:10.1159/000313949
59. Jones JM, Sapiano MRP, Savinkina AA, et al. Slowing decline in blood collection and transfusion in the United States - 2017. *Transfusion*. 2020;60(Suppl 2):S1–S9. doi:10.1111/trf.15604
60. Rossmann SN, Townsend M, Simon TL. Recruitment and screening of donors and the collection of blood. *Rossi's Princ Transfus Med*. 2022;2022:25–36.
61. Vamvakas E, Taswell H. Epidemiology of blood transfusion. *Transfusion*. 1994;34(6):464–470. doi:10.1046/j.1537-2995.1994.34694295059.x
62. Zou S, Musavi F, Notari EP, Fang CT. Changing age distribution of the blood donor population in the United States. *Transfusion*. 2007;071117010348009. doi:10.1111/j.1537-2995.2007.01517.x
63. Currie CJ, Patel TC, McEwan P, Dixon S. Evaluation of the future supply and demand for blood products in the United Kingdom National Health Service. *Transfus Med*. 2004;14(1):19–24. doi:10.1111/j.0958-7578.2004.00475.x
64. Liu W, Chen Y, Hsu L, Chen J, Wei S, Hou S. An imbalance in blood collection and demand is anticipated to occur in the near future in Taiwan. *J Formosan Med Assoc*. 2022;21(8):1610–1614. doi:10.1016/j.jfma.2021.07.027

65. Ali A, Auvinen MK, Rautonen J. Blood donors and Blood Collection: the aging population poses a global challenge for blood services. *Transfusion*. 2009;50(3):584–588. doi:10.1111/j.1537-2995.2009.02490.x
66. Ugwu A, Gwarzo D, Nwagha T, Gwarzo A, Greinacher A. Transfusion in limited infrastructure locations – where to go decades after safe blood initiative by World Health Organization? *ISBT Sci Ser*. 2020;15(1):118–125. doi:10.1111/voxs.12519
67. Erhabor O, Adias T, Mainasara A. Provisions of safe blood transfusion services in low income setting in West Africa, case study of Nigeria. *Adv Med Biol*. 2013;59:1–58.
68. Custer B, Zou S, Glynn SA, et al. Addressing gaps in international blood availability and transfusion safety in low-and middle-income countries: a NHLBI workshop. *Transfusion*. 2018;58(5):1307–1317. doi:10.1111/trf.14598
69. Sullivan HC, Roback JD. The pillars of patient blood management: key to successful implementation (Article, p. 2840). *Transfusion*. 2019;59(9):2763–2767. doi:10.1111/trf.15464
70. Leahy MF, Hofmann A, Towler S, et al. Improved outcomes and reduced costs associated with a health-system-wide patient blood management program: a retrospective observational study in four major adult tertiary-care hospitals. *Transfusion*. 2017;57(6):1347–1358. doi:10.1111/trf.14006
71. Leahy MF, Trentino KM, May C, Swain SG, Chuah H, Farmer SL. Blood use in patients receiving intensive chemotherapy for acute leukemia or hematopoietic stem cell transplantation: the impact of a health system-wide patient blood management program. *Transfusion*. 2017;57(9):2189–2196. doi:10.1111/trf.14191
72. Heitmiller ES, Hill RB, Marshall CE, et al. Blood wastage reduction using Lean Sigma methodology. *Transfusion*. 2010;50(9):1887–1896. doi:10.1111/j.1537-2995.2010.02679.x
73. Levin JH, Collins L, Adekunle O, et al. Blood product wastage reduction by utilising low-cost, low-impact multimodal physician-to-physician communication initiatives. *Transfus Med*. 2019;29(6):389–393. doi:10.1111/tme.12640
74. Morgan TO. Blood conservation: the CEO perspective. *J Cardiothorac Vasc Anesth*. 2004;18(4):S15–S17. doi:10.1053/j.jvca.2004.06.003
75. McKinney M. Maine hospital slashes transfusions, reducing patient risk, costs. *Mod Healthc*. 2014;25:30.
76. World Health Organization. *The Urgent Need to Implement Patient Blood Management: Policy Brief*. World Health Organization; 2021.
77. Stanworth SJ, New HV, Apolseth TO, et al. Effects of the COVID-19 pandemic on supply and use of blood for transfusion. *Lancet Haematol*. 2020;7(10):e756–e764. doi:10.1016/S2352-3026(20)30186-1
78. Flegel WA. COVID-19 insights from transfusion medicine. *Br J Haematol*. 2020;190(5):715–717. doi:10.1111/bjh.17005
79. Delabranche X, Kientz D, Tacquard C, et al. Impact of COVID-19 and lockdown regarding blood transfusion. *Transfusion*. 2021;61(8):2327–2335. doi:10.1111/trf.16422
80. Fede J, Hinrichsen C. Improving red blood cell usage at an academic medical center. *Medical Laboratory Observer*; 2022.
81. Jacobs JW, Karafin MS, Allen ES, et al. Blood conservation strategies at United States hospitals during the COVID -19 pandemic: findings from a multi-institutional analysis - International Society of Blood Transfusion survey. *Transfusion*. 2022;62(11):2271–2281. doi:10.1111/trf.17116
82. Kheirbeck T, Martin T, Wakely M, Lueckel S, Adams C. Safety and feasibility of ultra-restrictive transfusion protocol as a blood-preservation strategy during shortage crises. *R I Med J*. 2022;49–54. Available from: <http://www.rimed.org/rimedicaljournal/2022/09/2022-09.pdf#page=49>.
83. Doughty H, Green L, Callum J, Murphy MF. Triage tool for the rationing of blood for massively bleeding patients during a severe national blood shortage: guidance from the National Blood Transfusion Committee. *Br J Haematol*. 2020;191(3):340–346. doi:10.1111/bjh.16736
84. Beverina I, Ranzini M. A synchronized approach between emergency department and anemia clinic to intravenous iron treatment for very severe (Hb < 7.0 g/dL) and extreme (< 5.0 g/dL) iron-deficiency anemia: short-, medium- and long-term efficacy and safety analysis. *Blood Transfus*. 2023;21(3):235–239. doi:10.2450/2023.0199-22
85. Bolcato M, Russo M, Trentino K, Isbister J, Rodriguez D, Aprile A. Patient blood management: the best approach to transfusion medicine risk management. *Transfus Apher Sci*. 2020;59(4). doi:10.1016/j.transci.2020.102779

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Paroxysmal Nocturnal Hemoglobinuria: Current Management, Unmet Needs, and Recommendations

Monika Oliver & Christopher J Patriquin

To cite this article: Monika Oliver & Christopher J Patriquin (2023) Paroxysmal Nocturnal Hemoglobinuria: Current Management, Unmet Needs, and Recommendations, Journal of Blood Medicine, , 613-628, DOI: [10.2147/JBM.S431493](https://doi.org/10.2147/JBM.S431493)

To link to this article: <https://doi.org/10.2147/JBM.S431493>



© 2023 Oliver and Patriquin.



Published online: 05 Dec 2023.



Submit your article to this journal



Article views: 887



View related articles



View Crossmark data



Citing articles: 9 View citing articles

Paroxysmal Nocturnal Hemoglobinuria: Current Management, Unmet Needs, and Recommendations

Monika Oliver¹, Christopher J Patriquin² 

¹Department of Medicine, University of Alberta; Division of Hematology, University of Alberta Hospital, Edmonton, Alberta, Canada; ²Department of Medicine (Hematology), University of Toronto, Division of Medical Oncology & Hematology, University Health Network, Toronto, Ontario, Canada

Correspondence: Christopher J Patriquin, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario, M5G2C4, Canada, Tel + 1-416-340-5233, Fax + 1-416-340-3799, Email christopher.patriquin@uhn.ca

Abstract: Paroxysmal nocturnal hemoglobinuria (PNH) is an ultra-rare, acquired clonal abnormality, which renders hematopoietic cells exquisitely sensitive to complement-mediated destruction. Classical features of PNH include intravascular hemolytic anemia, increased thrombotic risk, and manifestations related to end-organ damage (eg fatigue, chest pain, dyspnea, renal failure, and pulmonary hypertension). With supportive care alone, mortality rate of patients with PNH is approximately 35%. The anti-C5 monoclonal antibody, eculizumab, was the first targeted therapy approved for PNH, and led to improved hemoglobin, quality of life, reduced transfusion need, reduced thrombosis, and greater overall survival. More recently, therapeutics such as longer acting anti-C5 (ravulizumab) and anti-C3 (pegcetacoplan) medications have been approved, along with other novel therapeutics in late-stage clinical trials. Biosimilars of eculizumab are also now available. Proximal inhibitors (against C3, factor B, and factor D) have shown significant improvements in hemoglobin and transfusion-avoidance in patients who remain anemic despite C5 inhibition. Despite these novel therapies, some unmet challenges remain, including management of breakthrough hemolysis, clinically significant iatrogenic extravascular hemolysis, optimal management in pregnancy, and infection risk mitigation as new targets in the complement system are blocked. In addition, the use of self-administered subcutaneous and oral therapies raises concerns around treatment adherence and the risks of uncontrolled terminal complement. Given the ultra-rare nature of PNH, development is underway of a centralized international registry to capture and analyze the data as they mature for various new therapies and characterize the clinical challenges related to PNH management.

Keywords: complement, hemolysis, hemolytic anemia, PNH, thrombosis

Introduction

PNH Pathophysiology and Disease Manifestations

Paroxysmal nocturnal hemoglobinuria (PNH) is an ultra-rare, acquired clonal abnormality of hematopoietic stem cells. The primary defect is a somatic mutation in the *PIGA* gene leading to the absence of glycosylphosphatidylinositol (GPI) anchors and, therefore, the GPI-anchor-bound molecules in hematopoietic cells. In PNH, the lack of GPI-linked regulatory proteins, CD55 (DAF – decay accelerating factor) and CD59 (MIRL – membrane inhibitor of reactive lysis), drive pathology. In the absence of these regulatory controls, complement activation on the surface of these cells can progress unchecked, culminating in formation of membrane attack complexes. This results in intravascular hemolysis (IVH) of vulnerable erythrocytes and activation of PNH leukocytes and platelets, manifesting in thrombosis risk, systemic morbidity, and pre-mature death.^{1,2}

PNH can affect individuals of any age group but is diagnosed most commonly in the third decade of life. There is no clear geographic, ethnic, or sex predilection. Manifestations of PNH are variable and include fatigue, dyspnea, abdominal pain, hemoglobinuria, and smooth muscle dystonia (eg esophageal spasms and erectile dysfunction). Symptoms are a consequence of unrelenting IVH and the complex interplay of complement and coagulation systems. Life-threatening complications of PNH include chronic kidney disease (CKD), pulmonary hypertension, as well as venous or arterial

thromboembolic events (TE).³ Historically, approximately, 20–35% of PNH patient would die within 5–10 years of diagnosis despite best available supportive care.¹ Thus, early detection is paramount to allow for prompt initiation of targeted therapy. PNH should be considered in any individual presenting with otherwise unexplained cytopenia, aplastic anemia or other bone marrow failure, atypical thrombotic presentation, Coombs-negative hemolysis, or hemoglobinuria.⁴ Diagnosis should be via high-sensitivity flow cytometry, to detect GPI-deficient populations of granulocytes, monocytes, and erythrocyte lineages.^{4,5}

Current Standard of Care

Standard of care (SOC) treatment for PNH varies broadly by country, with many nations still without any complement inhibition,⁶ though this may be improving as clinical trials of novel agents expand geographically. The only curative therapy for PNH is an allogeneic hematopoietic stem cell transplant (HSCT); however, as transplant-related morbidity and mortality are significant, HSCT is reserved for PNH patients with concomitant severe aplastic anemia or other causes of bone marrow failure (BMF), where HSCT is worth the risk of addressing both conditions.^{1,4,7} However, patients with a history of PNH-related thrombosis appear to do worse with HSCT and are best served by supportive care alone if targeted treatments are not available.^{8,9} As transplant medicine evolves with less intensive conditioning regimens and improved graft-versus-host disease and infectious prophylaxis, HSCT may eventually move up in the treatment algorithm, especially in jurisdictions where anti-complement therapies are not available. Use of eculizumab prior to transplantation does not appear to affect engraftment or increase transplant complication rates,¹⁰ and may protect against increased hemolysis when exposed to antithymocyte globulin.

Currently, the definitive treatment for hemolytic or thrombotic PNH is complement blockade. In Canada, as in many countries, first-line therapy is eculizumab or ravulizumab, monoclonal antibodies targeting terminal complement protein, C5, preventing its cleavage and participation in membrane attack complex formation. With C5 inhibition, IVH is substantially reduced, and major complications are prevented. Details regarding eculizumab and ravulizumab are presented later (see Terminal Complement Inhibition). The C3 inhibitor, pegcetacoplan, has also been given first-line indication in the US,¹¹ though it remains unclear how often it will be chosen before a trial of C5 blockade.

For all patients with PNH, supportive care is essential. This includes hematinic support with folic acid and iron supplementation in iron deficient patients. Transfusion support may be necessary for some patients with severe anemia and, in those patients, iron chelation may be required for transfusion-associated iron overload. Other adjuncts may be used, such as analgesia to manage abdominal pain and smooth muscle dystonia. Prophylactic anticoagulation may be considered for patients at highest thrombotic risk (eg granulocyte clone >30%, ≥ 2 HDA criteria, and prior TE),¹² only if they cannot be started promptly on complement inhibition; however, there are limited data to support this practice and thus a careful assessment of thrombotic and bleeding risk must be undertaken, in discussion with patients.^{4,13} Both eculizumab and ravulizumab carry a black box warning for life-threatening and fatal meningococcal infections. Thus, all patients require vaccination against meningococcal subtypes. Similar warnings exist for pegcetacoplan.¹¹ Anti-meningococcal antibiotics are required until 14 days post-vaccination, and some patients may elect to continue antimicrobial prophylaxis indefinitely.

Challenges & Unmet Needs

Despite the advances in PNH treatment over the last 20 years, leading to improved clinical outcomes, quality of life (QoL), and overall survival, significant challenges and unmet needs remain. Below, we have framed some of these as they relate to complications of current therapies, convenience of drug administration, and competition in the field of complement-mediated diseases.

Complications

One of the greatest challenges that occurs with current C5 inhibitors is persistent anemia and transfusion dependence. In the phase-3 trial comparing ravulizumab and eculizumab in complement inhibitor-naïve patients (Study 301), 26.4% on ravulizumab and 33.9% on eculizumab remained transfusion-dependent.¹⁴ Similarly, in the “switch” trial (Study 302 – ravulizumab versus eculizumab in eculizumab-experienced patients), approximately 24% in each arm did not achieve

stabilized hemoglobin (Hb).¹⁵ Excluding etiologies unrelated directly to PNH or its treatment,^{4,9,16} such as bleeding, bone marrow failure (BMF), and hematinic deficiencies, ongoing anemia despite C5 inhibition typically falls into two main categories: patients may have incomplete complement blockade and are susceptible to breakthrough hemolysis (BTH);^{17,18} or, patients may develop extravascular hemolysis (EVH) of the circulating GPI-negative erythrocytes no longer being hemolyzed intravascularly.^{19,20}

BTH is generally defined as an acute, recurrent elevation in LD plus at least one new or worsening manifestation of intravascular hemolysis (ie hemoglobinuria, fatigue, abdominal pain, TE, dysphagia, erectile dysfunction, or worsening anemia) after a patient has achieved control of IVH (ie LDH <1.5x ULN).²¹ It may be chronic or recurrent, secondary to insufficient complement inhibition nearing the end of each dosing cycle (*pharmacokinetic* [PK]-BTH) or regardless of C5 inhibition in the context of complement-amplifying conditions, such as infection or pregnancy (*pharmacodynamic* [PD]-BTH).^{18,22–24} A retrospective review of 14 eculizumab-treated patients (763 doses) identified 12 BTH episodes in four patients, most often occurring 24–48 hr before the next scheduled infusion.²⁵ In an analysis of 141 PNH patients treated with eculizumab for ≥13 months, 21% required a higher-than-label dose (ie ≥1200 mg) based on subtherapeutic eculizumab levels or unsuppressed CH50.²⁶ In the phase-3 randomized trials of ravulizumab versus eculizumab, those receiving ravulizumab had a more profound suppression of free C5 and, associated with this, no PK-BTH reported compared to four episodes in the eculizumab arm; PD-BTH occurred in a small number of patients in each group related to infections or other triggers.^{17,21} Rates and risks of BTH, and how to mitigate them, in patients receiving proximal inhibitors will require careful attention as the data across trials mature (see Proximal Complement Inhibition).

Treatment with C5 inhibition may result in EVH, as the CD55-negative circulating erythrocytes accumulate C3b and its split products. The diagnosis is assumed in the face of a normal or minimally elevated LD accompanied by reticulocytosis and C3d-positive direct antiglobulin test, the latter of which is observed in >50% of PNH patients treated with eculizumab.^{26,27} A retrospective analysis of 56 PNH patients receiving eculizumab found that 41 (73%) had significant C3 deposition on erythrocytes. The percentage of coated cells correlated with hematologic response, with optimal responders having the lowest C3 deposition.²⁰ These opsonized erythrocytes are tagged for destruction by the reticuloendothelial system and are ultimately hemolyzed within the liver and spleen. Importantly, clinically significant EVH only occurs once a patient is on effective C5 inhibition, and historical treatments including splenectomy or corticosteroids have been ineffective or associated with undue toxicity. In untreated PNH patients, low-level EVH may be theoretically present but is eclipsed by IVH.¹⁹ It is not yet possible to accurately identify those patients who may be at higher risk of developing clinically significant EVH, but a contribution of genetic variants in CR1 and complement factor H may play a role.^{28,29}

Even with a suboptimal response to eculizumab or ravulizumab (eg ongoing anemia ± transfusion-dependence), truly uncontrolled disease despite C5 inhibition is rare. Nishimura et al identified a C5 polymorphism in a minority of PNH patients in Japan (3.2%) at a prevalence similar to the Japanese general population (3.5%). This variant leads to a modified binding site such that eculizumab (and ravulizumab) cannot associate. As a result, patients with this C5 polymorphism (p.Arg885His) display frank drug resistance.³⁰

Another complication of therapeutic complement inhibition, which cannot be understated, is infection, particularly due to meningococcus and other encapsulated organisms. Assessing over a decade of post-marketing and pharmacovigilance data on eculizumab use in PNH, the meningococcal infection rate was 0.24 per 100 patient-years, based on a cumulative exposure of 21,016 patient-years.³¹ Patients require vaccination against meningococcal subtypes (eg ACWY and MenB) at initiation of therapy and every 3–5 years thereafter or based on titers if available; however, immunization alone may not be sufficient, as currently available vaccines do not cover all subtypes. Of the reported cases of meningococcal infection in patients receiving eculizumab in the United States from 2008 to 2016 (14/16 were ACWY-vaccinated), 11/16 *N. meningitidis* isolates were non-groupable.³² A similar risk exists for gonococcal infection, against which we cannot vaccinate, so careful sexual history and counselling should be done in advance of starting C5 inhibition.³³ As novel therapeutics are developed and as different steps in the complement cascade are inhibited, we will need to watch closely for signals of infection risk and work to mitigate them.³⁴

Convenience

Convenience of drug delivery and modality of treatment are also important limitations of currently approved complement inhibitors. Requiring indefinite fortnightly eculizumab infusions is burdensome to patients, caregivers, and healthcare system resources. Ravulizumab improves on this with its longer half-life (49.6 days versus 11.3 days) and is dosed every 8 weeks, allowing more flexibility for patients to travel and have more control of their schedules;^{14,15} however, intravenous delivery can still be challenging, especially for patients with needle phobias or suboptimal peripheral venous access. A minority may require a peripherally inserted central catheter (PICC) or other implanted device for their infusions.

Novel complement inhibitors have expanded options for delivery, including self-administered subcutaneous (SC) and oral therapies (see Treatment Pathways). SC molecules have variable treatment schedules ranging from multiple times per week,³⁵ to weekly,³⁶ or monthly.^{37–40} Oral monotherapies currently being tested must be taken twice a day.^{41,42} Assuming commercial approvals, these options would allow patients to deliver their own treatment, but must be weighed against the risk of poor compliance or inability to tolerate oral intake (eg gastroenteritis), which would expose them to worsening IVH and potential complications, such as TE. The severity of a BTH episode is also potentially increased, with more profound resultant anemia, in patients with larger type-III erythrocyte clones.¹⁷ Another consideration is the strict storage requirements for some therapies (eg refrigeration for pegcetacoplan and iptacopan) that may have significant implications for travel. Finally, the potential for drug–drug interactions, not an issue with eculizumab or ravulizumab, would present potential limitations in prescribing and management of comorbid conditions.⁴³

Competition

Widespread access to anti-complement therapy remains a significant challenge. This is driven in large part by the high costs of current SOC therapies, and the economic burden to health-care systems.^{44–46} Competition in the complement inhibitor space is expected to drive down the benchmark prices of current therapies, while encouraging responsible price listings for new molecules. Prior to commercial listing, multiple industry partners running trials in the same area were driving more rigorous trial methodology, and a more nuanced understanding of the complement system and its inhibition. The latter has the potential to drive the innovation of novel targets to treat not only PNH but also other complement-mediated disorders.⁴⁷ The frenzy of PNH clinical trials over the past decade has also brought treatment options to countries without prior access to therapies.^{37,48–50} We remain hopeful that this will result in wider availability and hopefully global reach of effective PNH therapies.

Treatment Pathways

It has now been over 20 years since eculizumab was first used to treat patients with PNH,⁵¹ and the field since then has expanded significantly. This is exciting for patients and physicians alike; however, at the same time, many more treatment options can also cause confusion when making clinical decisions. Below, we review the various pathways for therapeutic complement inhibition, focusing on molecules already approved or in late-stage clinical development (see Table 1).

Terminal Complement Inhibition

Eculizumab was the first inhibitor of terminal complement to be developed for clinical use, and even though its benefit in the initially intended indications was underwhelming,⁴⁷ it has become clear that terminal complement inhibition is highly effective in controlling IVH, and protecting patients from thrombotic and other complications.⁵² However, approximately 40% of patients receiving eculizumab suffer from significant residual anaemia (Hb <100 g/L) and many remain transfusion dependent.⁵³ It became clear that blocking C5 created the situation of iatrogenic extravascular hemolysis (EVH) due to the persistent circulation of CD55-negative erythrocytes. There are many novel C5 inhibitors, but all are expected to have the same potential for EVH.¹⁹

Eculizumab and Ravulizumab

Eculizumab is a fortnightly IV infusion first approved for PNH in 2007. It was the first targeted therapy to show efficacy in the disease, with now over two decades of experience since the first patients enrolled in the pilot study.⁵¹ The landmark phase-3 trials (TRIUMPH [N = 87] and SHEPHERD [N = 97]) showed improvement in Hb and transfusion

Table 1 Complement Inhibitors Approved or in Late-Stage Clinical Trial for Treatment of PNH

Treatment Strategy	Name	Molecular Target	Compound Type	Modality & Frequency ^a	Indication(s) ^b
Proximal Inhibition	Pegcetacoplan	C3	Pegylated cyclic peptide	1080mg SC twice weekly (increased frequency every 3 days or thrice weekly can be considered) IV x1 or SC daily x 3 days	Hb <105 g/L despite 3–6 months of C5i Complement inhibitor-naïve (US only) BTH (based on a small study to date and not a formal trial)
	Iptacopan	Factor B	Small molecule	200mg PO BID	Hb <100 g/L despite stable C5i for ≥6 months Complement inhibitor-naïve
	Vemircopan	Factor D	Small molecule	120–180mg PO BID	Hb <105 g/L despite stable C5i for ≥6 months Complement inhibitor-naïve
	BXC9930	Factor D	Small molecule	500 mg PO BID	Complement inhibitor-naïve or switch from C5i
Terminal Inhibition	Eculizumab	C5	Monoclonal antibody	IV 600mg weekly x 4 weeks, followed by 900mg week 5 and every 2 weeks N.B. Dose increases to ≥1200mg every 2 weeks may be required	For treatment-naïve PNH patients with elevated LD >1.5x ULN and at least one disease manifestation (refer to local approval criteria for specifics) New thrombosis related to PNH, regardless of degree of hemolysis
	Ravulizumab	C5	Monoclonal antibody	Weight 40kg to <60kg Loading: 2400mg x1 Maintenance: 3000mg 2 weeks after loading dose, then every 8 weeks Weight 60kg to <100kg Loading: 2700mg x1 Maintenance: 3300mg 2 weeks after loading dose, then every 8 weeks Weight ≥100kg Loading: 3000mg x1 Maintenance: 3600mg 2 weeks after loading dose, then every 8 weeks SC 490mg weekly 2 weeks after IV loading dose (see above)	Same as for eculizumab N.B. Use in pregnancy remains a case-by-case decision where available

(Continued)

Table 1 (Continued).

Treatment Strategy	Name	Molecular Target	Compound Type	Modality & Frequency ^a	Indication(s) ^b
	Crovalimab	C5	Monoclonal antibody	Weight 40kg to 100kg Loading: 1000mg IV on week 1 (day 1) followed by 340mg SC on week 1, day 2, and weeks 2–4 Maintenance: 680mg SC week 5 and every 4 weeks Weight ≥100kg Loading: 1500mg IV on week 1 (day 1) followed by 340mg SC on week 1 (day 2), and weeks 2–4 Maintenance: 1020mg SC week 5 and every 4 weeks	Complement inhibitor-naïve Treated with eculizumab or ravulizumab for ≥3 months, with LD ≤2x ULN
	Tesidolumab	C5	Monoclonal antibody	IV 20mg/kg every 2 weeks	Complement-inhibitor naïve (including patients with C5 polymorphism)
	Pozelimab + cemdisiran	C5	Monoclonal antibody (pozelimab) + siRNA (cemdisiran)	Pozelimab Loading: 30mg/kg IV x 1 followed by 400mg SC x 1. Maintenance: 400mg SC every 4 weeks after loading complete Cemdisiran Loading: 200mg SC x 1 Maintenance: 200mg SC every 4 weeks after loading complete	Complement-inhibitor naïve (including patients with C5 polymorphism)
	ABP959 ^c	C5	Monoclonal antibody	Same as for eculizumab	Same as for eculizumab
	SB12 ^c	C5	Monoclonal antibody	Same as for eculizumab	Same as for eculizumab
	Elizaria ^c	C5	Monoclonal antibody	Same as for eculizumab	Same as for eculizumab
Dual Inhibition	Danicopan + eculizumab or ravulizumab	Factor D + C5	Small molecule + monoclonal antibody	150–200mg PO TID + eculizumab or ravulizumab, as prescribed	Persistent anemia (<95 g/L) despite eculizumab or ravulizumab

Notes: ^aAdministration route and frequency provided reflect maintenance dosing. Please refer to trial protocols and product monographs for loading-dose information. ^bIndications provided reflect either those used for commercial drug availability or, if not yet approved, the clinical trial enrolment criteria. Please refer to local health authorities for updated information. ^cBiosimilars based on eculizumab (Soliris) as the reference product.

Abbreviations: BID, bis in die (2 times daily); BTH, breakthrough hemolysis (see text for definition); C5i, C5 inhibition; Hb, hemoglobin; IV, intravenously; LD, lactate dehydrogenase; N.B., nota bene; PO, per os (oral treatment); SC, subcutaneously; siRNA, small inhibitor RNA; TID, ter in die (3 times daily); ULN, upper limit of normal of lab reference.

independence, reduction in thrombotic events, recovery of renal function, plus improved fatigue and overall QoL.^{54–58} Subsequent analysis demonstrated safe and effective use of eculizumab in pregnant patients as well.²⁴

Ravulizumab, like eculizumab, inhibits C5 but offers a half-life approximately four-fold longer, achieved due to a 4-amino-acid substitution; this allows off-loading of C5 in the endosome and recirculation via the neonatal Fc receptor (FcRn).⁵⁹ Ravulizumab is typically administered by intravenous infusion every 8 weeks, with a weight-based regimen. Efficacy of ravulizumab was shown in two Phase 3 clinical trials (Study 301 [N = 246] and Study 302 [N = 197]).^{14,15} The primary endpoints of LD improvement or normalization, and transfusion avoidance were achieved. Ravulizumab is noninferior to eculizumab with respect to four key secondary endpoints as well, including the proportion of patients with BTH, Hb stabilization, and improvement in FACIT-fatigue. Subsequent analysis of BTH demonstrated no occurrence of PK-BTH in the ravulizumab arms,²¹ consistent with the more profound inhibition of free C5.⁵⁹ Though data need to mature, overall survival for patients receiving ravulizumab also seems similar to those on eculizumab.⁶⁰ Weekly subcutaneous ravulizumab given via an on-body delivery system has shown similar efficacy, safety, and patient preference,³⁶ and could serve as an alternative to infusions, particularly in situations of poor venous access or during extended travel.

Crovalimab

Crovalimab is a novel C5 inhibitor developed using soluble monoclonal antibody recycling technology (SMART) which, like ravulizumab, offloads bound C5 in acidified endosomes and employs FcRN recycling to extend half-life.⁶¹ Following an initial IV loading dose, it is given subcutaneously (SC) every 4 weeks. The COMPOSER trial was a phase-1/2 study of 44 patients who were either naive to treatment or switched from eculizumab at enrolment. It reported continued hemolysis control, Hb stabilization, and transfusion avoidance, with five BTH events reported during the open-label extension phase. Each BTH event occurred in one individual; three of these were temporally associated with an infectious complication, and all resolved with continuation of crovalimab.⁶² The phase-3 COMMODORE-3 trial, conducted in China, enrolled transfusion-dependent, treatment-naive patients (n=51) with elevated LD (>2× upper limit of normal [ULN]). Both co-primary efficacy endpoints were met, including hemolysis control (LD <1.5× ULN) in 78.7% (95% CI: 67.8–86.6) and transfusion avoidance in 51%.³⁷ Results from the other phase-3 trials, COMMODORE-1³⁹ and COMMODORE-2,³⁸ have recently been reported for patients who were either switched from SOC or were treatment-naive. Similar control of hemolysis and transfusion needs were seen. Of note, in the switch trial, 16% of crovalimab-treated patients had drug-target-drug complex (DTDC)-mediated Type III hypersensitivity events occur due to the two anti-C5 antibodies targeting separate binding sites. Most events were mild-to-moderate, isolated to skin and joints, and resolved with no change in crovalimab dosing.³⁹

Pozelimab + Cemdisiran

A phase-3, randomized trial of the anti-C5 monoclonal, pozelimab,⁶³ plus cemdisiran,⁶⁴ compared to SOC (ravulizumab or eculizumab) is currently recruiting at the time of writing. In addition to directly inhibiting C5 with pozelimab, the use of cemdisiran, a small interfering RNA (siRNA), should further reduce terminal complement activity by directly suppressing hepatic C5 synthesis. Cemdisiran alone has shown a >90% reduction of C5 production in patients with PNH; however, this was insufficient to fully inhibit complement activation alone. The two-drug combination, as part of a phase-2 trial, demonstrated successful control of terminal complement, with an interim analysis (n=16) demonstrating that most participants maintained normalization of LD, and 75% achieved Hb stabilization.⁴⁰ Results of the phase-3 trial are anticipated.

Tesidolumab

Tesidolumab is a fully human anti-C5 monoclonal antibody, currently used mostly in Asia. Like crovalimab and pozelimab, it too binds to a distinct epitope from eculizumab and ravulizumab, permitting use in those with C5 polymorphisms.³⁰ A phase-2 trial of nine patients, five of whom carrying the Arg885His C5 variant, demonstrated approximately 80% reduction in LD levels, decreased transfusion dependence, a 20 g/L mean Hb increase, and improvement in FACIT-fatigue scores.⁶⁵ Further, publicly available data are awaited.

Eculizumab Biosimilars

There are currently three eculizumab biosimilars,⁶⁶ and their reduced cost should allow for expanded availability of complement inhibition in jurisdictions currently still without these life-saving therapies. Two of the molecules, ABP-959^{67,68} and SB12^{49,69} are either recently available in some countries or are in the process of being assessed by national health authorities. They have shown comparable efficacy, safety, and immunogenicity to eculizumab as a reference product. Elizaria is another eculizumab biosimilar, currently marketed in Russia, and is a first-line therapy for patients with PNH. Those previously on eculizumab were subsequently switched once it was available.⁷⁰ A phase-3 trial confirmed non-inferiority compared to eculizumab with respect to LD control, secondary outcomes, and safety parameters.⁴⁸ How these biosimilars, given fortnightly, will fit into health systems that already have ravulizumab remains to be seen, but could at least provide a more affordable option for the many countries still without C5 inhibition for PNH. To our knowledge, there are no biosimilars that are currently longer acting.

Proximal Complement Inhibition

Interest in proximal complement inhibition as a therapeutic strategy stems from the need to correct the iatrogenic C3-mediated EVH that can occur in PNH patients on a C5 inhibitor. Much of the early work came from the Lambris lab with the C3 inhibitor, compstatin.⁷¹ Its pegylated version, pegcetacoplan, was the first such therapeutic approved for use in patients with PNH. Three main classes of proximal inhibitor exist and are either approved for use or are in late-stage clinical trials, blocking C3, factor D (FD), and factor B (FB).⁷²

C3 Inhibition

C3 inhibition is the first successful strategy to treat PNH since terminal complement inhibitors were approved. Pegcetacoplan, a pegylated anti-C3 cyclic peptide, given as a twice-weekly SC infusion, demonstrated superiority in change from baseline Hb (mean difference 38.4 g/L) compared to eculizumab in a randomized, open-label clinical trial (PEGASUS) of patients who had persistent anemia (Hb <105 g/L) despite eculizumab, many of whom were transfusion-dependent.³⁵ It was found to be non-inferior in terms of transfusion-avoidance and change from baseline reticulocyte count, but not for change from baseline LD. Due to hierarchical statistical analysis, QoL was not formally analyzed; however, a post hoc analysis found pegcetacoplan superior to eculizumab across FACIT-fatigue domains.⁷³

Following PEGASUS, PRINCE was an open-label, controlled trial completed in complement inhibitor-naïve patients, compared to supportive care alone (ie no C5 inhibition).⁵⁰ Pegcetacoplan was superior with respect to Hb stabilization (85.7% vs 0% in the SOC arm) and hemolysis control, with a mean LD at 26 weeks of 205 U/L vs 1535 U/L (ULN = 226 U/L). Patients in any of the parent pegcetacoplan trials were eligible to be enrolled in the APL2-307 open-label extension study of pegcetacoplan and demonstrated durable improvements in Hb, transfusion avoidance, LD, and FACIT-fatigue scores.⁷⁴

A key finding of the pegcetacoplan trials was a significant expansion of GPI-negative erythrocyte populations, often exceeding 90%. This was associated with the increased Hb seen; however, in PEGASUS, episodes of acute hemolysis occurred in four patients and were dramatic, with LD increasing to 10–15x ULN in some cases, and 3 out of the 4 participants discontinued pegcetacoplan.³⁵ Recognition of the potential for profound BTH prompted a sub-study of those in the open extension period, in which those with acute hemolysis could receive either a single IV dose, or three consecutive-day doses of SC pegcetacoplan, followed by every-3-days dosing. Preliminary data suggest that this strategy can successfully manage BTH in many patients on pegcetacoplan,⁷⁵ though some may benefit from temporary use of C5 inhibitors.⁷⁶ In the APL2-307 extension trial, 16.8% of patients developed an acute hemolytic episode. All of these resolved, though 7/9 received increased dosing and three required transfusions. Three of these patients elected to discontinue trial participation due to hemolysis.⁷⁴ A clear and stratified approach on how best to manage BTH for physicians will be essential.

Factor B Inhibition

Factor B is an enzyme only active in the alternative pathway of complement and is required to form the alternative-pathway convertases of C3 and C5. Targeting FB theoretically permits ongoing functionality of the classical and lectin pathways. Iptacopan is an orally available, small-molecule FB inhibitor being trialed in multiple complement-mediated conditions. Its activity in PNH was first demonstrated as an add-on therapy for those still anemic despite C5 inhibition.⁷⁷

In the phase-3 APPLY-PNH trial of anemic patients (n=97) despite eculizumab, twice daily iptacopan monotherapy was associated with a mean Hb increase of 35.9 g/L. Only two patients in the iptacopan arm received transfusions versus 60% of those who stayed on eculizumab.⁴² The APPOINT-PNH trial is a single-arm study of complement inhibitor-naïve patients (n=40) with baseline Hb <100 g/L. In a press release, Novartis announced that, at 24 weeks, 92.2% of patients had a ≥ 20 g/L increase in Hb, 62.8% had a Hb of 120 g/L or more, and 95% had LD below 1.5x ULN.^{78,79}

Factor D Inhibition

Factor D is synthesized primarily in adipose tissue and circulates at the lowest concentration of all complement proteins.⁸⁰ This is the rate-limiting step of the alternative pathway, as it needs to cleave FB for it to partake in convertase activity with C3b or C3H₂O. As with FB blockade, inhibition of FD impairs alternative pathway complement function while allowing other pathways to function. Danicopan, discussed in more detail later (see Dual-Target Inhibition), was the first FD inhibitor to show activity in PNH patients and provided a proof of concept. Since then, vemircopan and BCX9930 have moved forward in trials, used as oral monotherapy.

Vemircopan, taken twice daily, is advancing through a phase-2 clinical trial program with three cohorts, including those who have switched from danicopan monotherapy, those with suboptimal response to C5 inhibition, and treatment-naïve patients. In the last group, 54.5% of whom were receiving transfusions during screening, vemircopan was associated with a mean Hb increase of 39 g/L, 81% reduction in LD, clinically significant improvement in FACIT-fatigue scores, and only one patient required transfusion on day 2 of treatment.⁴¹ Updated data and results from the other cohorts are anticipated.

BCX9930 is another twice-daily oral FD inhibitor that was being tested in patients with PNH who were either suboptimal responders to C5 inhibition or were treatment-naïve. Those receiving the drug showed improved Hb and hemolytic parameters and reduced transfusion requirements.^{81,82} Due to concerns related to crystalline nephropathy, potentially limiting dose optimization, the program was discontinued; however, patients already on BCX9930 and benefiting from it have continued. In a press release, BioCryst announced that they are in the early stages of developing a once-daily FD inhibitor (BCX10013) for another attempt at treating PNH and other complement-mediated diseases.⁸³

Dual-Target Inhibition

The current rationale for dual-target complement inhibition in PNH is to maintain a direct blockade of C5 while also providing proximal inhibition to protect against or treat EVH. Proximal inhibition would in essence allow for greater increments in Hb, while terminal blockade could protect against severe episodes of BTH in patients with significant proportions of circulating type-III erythrocytes.¹⁷

In eculizumab-treated PNH patients who remained transfusion-dependent, presumably due to EVH, the addition of thrice-daily danicopan (anti-FD) in a phase-2 trial was associated with a mean Hb increase of 24 g/L and a significant reduction in transfusion needs.⁸⁴ The phase-3 ALPHA trial of the same combination has completed enrolment, with full results anticipated soon.⁴³ Rapid increases in Hb were similarly seen in the PEGASUS trial during the 4-week run-in period, where participants received both pegcetacoplan and eculizumab.³⁵ A trial of KP104, a bifunctional monoclonal antibody that inhibits C5 while employing complement factor H activity for proximal pathway regulation, has recently started in patients with PNH, with data eagerly awaited.⁸⁵

A combined-target approach to treating PNH is certainly intriguing, and the data thus far suggest that it could be successful. The main limiting factor will be economic. This could be possible if both therapeutics are from the same company or, in the example of KP104, a single agent has multiple targets; however, given the extreme prices of orphan drugs, even modest incremental cost may be infeasible. Beyond pricing, questions remain surrounding who would benefit most, if combination therapy is required long term or for certain periods, and which combinations are most safe and effective.

Management Recommendations

The goal of the International PNH Interest Group (IPIG - <https://www.pnhinterestgroup.org/>) and other organizations is to continue to advocate for effective therapies for PNH patients internationally. Considering this important goal, we have

outlined some clinical scenarios and topics that may be of help to physicians who may not manage PNH on a regular basis. We would also encourage the reader to refer to national standards and guideline documents where available.

Initiating Therapy

In patients with small clones (usually granulocyte populations <10%) with no evidence of hemolysis or PNH-related disease manifestations, anti-complement therapy may not be required and it may be appropriate to closely monitor with blood work and clinical assessments. Close monitoring and supportive care alone may also be sufficient in patients with larger clones who do not otherwise meet indications for targeted therapy.

In patients with clinically significant hemolytic PNH, control of terminal complement is central, irrespective of the therapeutic target chosen. This is also the case for thrombotic PNH which can in some cases present without significant hemolysis, particularly if there is a predominance of type-II erythrocytes.⁸⁶ Though this may change soon, in Canada we remain limited to eculizumab and, in some situations, ravulizumab. Many jurisdictions use high disease activity (HDA)⁸⁷ criteria for approval decisions, permitting any PNH patient with LD >1.5x ULN and at least one manifestation of the disease to receive therapy. Some countries, such as Canada, may be limited by more restrictive criteria that are set by national HTA bodies.^{4,88} In addition to standard approval criteria, pregnancy in a patient with PNH is another indication to start eculizumab, even if they were not receiving C5 inhibition prior to conception, given the increased risk during this time.²⁴ In some urgent situations, such as new TE or severe hemolysis with end-organ damage (eg acute kidney injury), C5 inhibition must be started urgently to prevent progression. The rapidity of terminal complement blockade is crucial in these scenarios. As such, we would not use subcutaneous therapies, which can take longer to reach therapeutic levels.⁸⁹ Particularly with these presentations, we would not delay starting drug due to vaccination, and would cover patients with anti-meningococcal prophylaxis until it is possible and safe to vaccinate (see Mitigating Infection Risk).⁴ In the case of acute thrombosis, therapeutic anticoagulation is strongly recommended. There is little data to guide choice or duration of anticoagulation for TE in the setting of PNH, and it would depend in part on the site and severity of the TE presentation; however, we will routinely use direct oral anticoagulants (DOACs) for a minimum of 3–6 months and until control of IVH is achieved after starting anti-complement therapy. We engage in shared decision-making with patients thereafter, to weigh recurrent thrombotic versus bleeding risk factors to determine the need for ongoing prophylaxis thereafter.

At the time of writing, based on the evidence available, we would suggest starting patients on C5 inhibition in the upfront setting, with ravulizumab being preferred due to convenience and lower risk of PK BTH.²¹ This may change over time, as naïve-start data for other therapeutics mature; the PRINCE and APPOINT-PNH data suggest that pegcetacoplan and iptacopan, respectively, are also safe and effective therapies to start in complement inhibitor-naïve individuals.^{50,79} With access to multiple anti-complement therapies, patient preference will be a key consideration. In jurisdictions with limited access, we would favour initiating complement blockade in any form available given the risks of untreated PNH.^{1,52}

Whom to Switch

Patients who have ongoing anemia attributed to clinically significant EVH, particularly if symptomatic or transfusion-dependent, would be the optimal candidates to be switched to proximal inhibition. The choice of therapeutics will depend on patient preference, clinical characteristics, and jurisdictional availability. Most therapies recently approved or in late-stage development have clinical trial data for patients switching from SOC C5 inhibition. At baseline, patients who meet the characteristics of trial participants are those whom we would consider switching (eg persistent anemia despite 3–6 months of C5 inhibition).

Criteria to gauge hematologic response have been proposed, which consider Hb, LD, and reticulocyte count.¹⁹ A subsequent multicentre review (n=160) found that 21.3% had a complete response (normal Hb, transfusion-independent), 40.2% had a good response (Hb 100–120 g/L, transfusion-independent), with the remainder achieving only partial (26.8%) or minor (11.8%) response.⁵³ However, these criteria have been criticized for valuing an increased Hb over LD, a marker of IVH (eg a “major response” would permit LD >1.5x ULN, as long as the Hb is over 120 g/L).⁹⁰ Being permissive of LD >1.5x ULN could place patients at risk of TE, as previous studies have shown a direct association between elevated LD and thrombosis.⁹¹ Another concern is that regulators and payers could withdraw life-saving therapies

if improvement in Hb is weighted too heavily, without consideration of LD control. As such, a re-evaluation of response criteria is planned.

With respect to switching treatments, there are further considerations, not least of which is patient preference and willingness to change. As deep and consistent complement blockade is necessary to protect patients from TE and end-organ damage, additional issues such as adherence to oral therapies^{41,42,92} or challenges with self-administered delivery mechanisms (eg syringes and infusion pumps)^{35–37,62,93} need to be carefully considered.

Based on the data thus far, those with a significant thrombotic history seem best served by staying on a C5 monoclonal antibody; in particular, there are no data that we are aware of to support starting a novel therapeutic in a patient presenting with de novo TE. Lastly, we must be very cautious in the context of pregnancy since pregnant and nursing patients were explicitly excluded from trials. For now, we would recommend remaining on (or switching to) eculizumab during pregnancy given the long-term experience and data demonstrating its beneficial use,²⁴ though there are different practices and opinions, and this may change as safety data become available.

Mitigating Infection Risk

All patients receiving complement inhibition to treat PNH require meningococcal vaccination, with both quadrivalent and serogroup B formulations where available. As vaccines can amplify complement activity and lead to acute hemolysis,⁹⁴ it is our standard practice to first start complement inhibition and administer vaccines thereafter, providing antimicrobial prophylaxis for at least 14 days after they are given. For long-term protection, we provide boosters for each vaccine every 3 years (maximum, every 5 years).⁴ Others, with the assay available, will revaccinate at a variable frequency based on anti-meningococcal titres. Pneumococcal and Haemophilus type B vaccinations are recommended for patients receiving proximal inhibitors. Despite concerns that blocking “higher up” the complement cascade could increase infection risk, this has not been seen to date in the data available. Indeed, response to meningococcal vaccination was no worse in those patients receiving danicopan compared to those on eculizumab.^{95,96} As data mature for other therapies, we will need to watch vigilantly for any safety signals of increased or differential infection risk.

Long-term prophylactic antibiotics in addition to vaccination are recommended in several countries, but not all. The most common regimen is penicillin twice-daily, or possibly azithromycin in the case of penicillin allergy.⁴ An analysis of the International PNH Registry did not find any significant difference in rate of meningococcal infection between those who did and did not take prophylactic antibiotics (0.1 per 100 patient-years), but the analysis was limited by lack of a standard definition and duration of “prophylactic” and low meningococcal infection event rates.⁹⁷ The decision to take long-term antibiotics depends on patient values and preferences, and the possibility of side effects. Irrespective of prophylactic antimicrobial use, many experts suggest a “pill in pocket” approach, in which patients fill a prescription (typically of ciprofloxacin) with instructions to take a dose at the first signs of meningitis or unexplained fever and proceed to their closest emergency department. This is particularly important for patients who may live in remote areas or when abroad. Use of eculizumab without immunization has been done and reported safe, but only in the context of HSCT-associated thrombotic microangiopathy, during the discrete period before immune reconstitution.⁹⁸ It is rare to have a patient with PNH unable to receive vaccines; however, a discussion of the risks and benefits of complement inhibition without immunization would need to occur, and antimicrobial prophylaxis would be necessary.

Pregnancy

Management of PNH patients who become pregnant remains an important focus. Without complement inhibition, maternal mortality, mostly driven by thrombotic complications, ranged from 8% to 20% historically.²⁴ There is now many years’ experience using eculizumab in this context, with improved maternal outcomes and acceptable maternal and neonatal safety profile.²⁴ For our pregnant patients, we also recommend low molecular weight heparin prophylaxis during pregnancy and for 6 weeks post-partum, as well as early initiation of aspirin to protect against preeclampsia. All of these patients are managed alongside colleagues in high-risk obstetrics. There is growing experience using ravulizumab in pregnancy, but few published reports; the implications of greater reliance on the FcRn on the fetus require further attention. The safety of other complement inhibitors remains to be seen, and there is a plan in place to do this centrally within the IPIG Registry.

Where are We Going?

The PNH treatment landscape is evolving. With new treatments on the horizon, patients and physicians alike are excited about the trajectory and momentum of this evolution. Unfortunately, targeted anti-complement agents are still available in only a minority of countries.⁶ With increased competition in terms of PNH treatments, we are hopeful that the cost of SOC therapy will be reduced; however, as with most orphan drugs, we anticipate that costs will continue to be high.^{45,46,99} This will create challenges for resource-limited countries and may result in delays in drug approval.

As new therapies become available, and hopefully have broader geographic spread, it will be important to capture efficacy and safety data comprehensively. The current International PNH Registry (NCT01374360) was established in 2007, when eculizumab was the only treatment available and has been an invaluable resource to advance our knowledge of PNH and its management. A new registry, under the auspices of the International PNH Interest Group, is planned to start in the near future and will aim to enroll PNH patients receiving therapeutics by any company that participates, as well as those patients not on complement inhibition.

As the field progresses, and long-term extension data for newer therapies mature, prescribers will require a roadmap to help navigate the anti-complement space, including direction on initiation in treatment-naïve patients, whom and when to switch, and how to manage specific situations like BTH, TE, and pregnancy. International guidelines that will set standards for overall diagnostic and management strategies, while accounting for jurisdictional variation, will be an invaluable resource. Guidance should also come from local consensus guidelines, which consider the available therapies and nuances in each region. Physicians and patients will need to engage in shared decision-making to determine which therapy fits their disease characteristics and lifestyle needs. Drug availability, compliance, and modality of delivery will be important pillars of these conversations.

While the gaps in the PNH treatment landscape are reducing, there remain several unmet needs as we have outlined here. A more nuanced and precision medicine-based understanding of PNH could identify patients at particular risk of incomplete IVH control or clinically significant EVH with C5 inhibition. This information may allow us to risk-stratify patients at the time of diagnosis, and guide whether distal or proximal complement inhibition is most appropriate in the front-line setting, and which proximal target would be most effective. Lastly, additional complement biomarkers would be welcome, allowing a clearer distinction between IVH and EVH, and more objective targets to guide when a switch in therapy should be considered.¹⁰⁰ We have learnt much over the last 20 years since targeted complement inhibition first became possible, with great strides toward improvements in patient care. Given the ultra-rare nature of PNH, ongoing success now and in the future will come from continued and expanded international collaboration.

Author Contributions

Both authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

Dr Monika Oliver reports personal fees from Alexion, Sobi, Novartis, Takeda, and Sanofi, outside the submitted work. Dr Christopher Patriquin reports personal fees from Alexion, BioCryst, Amgen, Roche, Novartis, Sobi, Sanofi, and Takeda; Global PI for PNH clinical trial for Regeneron, outside the submitted work. The authors report no other conflicts of interest in this work.

References

1. Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 1995;333(19):1253–1258. doi:10.1056/NEJM199511093331904
2. Brodsky RA. Narrative review: paroxysmal nocturnal hemoglobinuria: the physiology of complement-related hemolytic anemia. *Ann Intern Med*. 2008;148(8):587–595. doi:10.7326/0003-4819-148-8-200804150-00003
3. Schrezenmeier H, Muus P, Socie G, et al. Baseline characteristics and disease burden in patients in the international paroxysmal nocturnal hemoglobinuria registry. *Haematologica*. 2014;99(5):922–929. doi:10.3324/haematol.2013.093161

4. Patriquin CJ, Kiss T, Caplan S, et al. How we treat paroxysmal nocturnal hemoglobinuria: a consensus statement of the Canadian PNH Network and review of the national registry. *Eur J Haematol*. 2019;102(1):36–52. doi:10.1111/ejh.13176
5. Borowitz MJ, Craig FE, Diguseppe JA, et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. *Cytometry B Clin Cytom*. 2010;78(4):211–230. doi:10.1002/cyto.b.20525
6. Partnering4PNH. A global policy consensus paper to address remaining unmet medical needs for people living with PNH; 2022. Available from: <https://pnh.sobi.com/sites/default/files/P4PNH-consensus-paper-short-version%20-%2015.12.2022%20.pdf>. Accessed December 02, 2023.
7. Brodsky RA. Stem cell transplantation for paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2010;95(6):855–856. doi:10.3324/haematol.2010.023176
8. Peffault de Latour R, Schrezenmeier H, Bacigalupo A, et al. Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2012;97(11):1666–1673. doi:10.3324/haematol.2012.062828
9. Risitano AM, Peffault de Latour R. How we (‘ll) treat paroxysmal nocturnal haemoglobinuria: diving into the future. *Br J Haematol*. 2022;196(2):288–303. doi:10.1111/bjh.17753
10. Vallet N, de Fontbrune FS, Loschi M, et al. Hematopoietic stem cell transplantation for patients with paroxysmal nocturnal hemoglobinuria previously treated with eculizumab: a retrospective study of 21 patients from SFGM-TC centers. *Haematologica*. 2018;103(3):e103–e105. doi:10.3324/haematol.2017.182360
11. Heo YA. Pegcetacoplan: a review in paroxysmal nocturnal haemoglobinuria. *Drugs*. 2022;82(18):1727–1735. doi:10.1007/s40265-022-01809-w
12. Hochsmann B, Peffault de Latour R, Hill A, et al. Risk factors for thromboembolic events in patients with paroxysmal nocturnal hemoglobinuria (PNH): a nested case-control study in the International PNH Registry. *Ann Hematol*. 2023;102(11):2979–2988. doi:10.1007/s00277-023-05402-3
13. Griffin M, Munir T. Management of thrombosis in paroxysmal nocturnal hemoglobinuria: a clinician’s guide. *Ther Adv Hematol*. 2017;8(3):119–126. doi:10.1177/2040620716681748
14. Lee JW, Sicre de Fontbrune F, Wong Lee Lee L, et al. Ravulizumab (ALXN1210) vs eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study. *Blood*. 2019;133(6):530–539. doi:10.1182/blood-2018-09-876136
15. Kulasekararaj AG, Hill A, Rottinghaus ST, et al. Ravulizumab (ALXN1210) vs eculizumab in C5-inhibitor-experienced adult patients with PNH: the 302 study. *Blood*. 2019;133(6):540–549. doi:10.1182/blood-2018-09-876805
16. Kulasekararaj AG, Brodsky RA, Hill A. Monitoring of patients with paroxysmal nocturnal hemoglobinuria on a complement inhibitor. *Am J Hematol*. 2021;96(7):E232–E235. doi:10.1002/ajh.26176
17. Notaro R, Luzzatto L. Breakthrough Hemolysis in PNH with Proximal or Terminal Complement Inhibition. *N Engl J Med*. 2022;387(2):160–166. doi:10.1056/NEJMra2201664
18. Brodsky RA. How I treat paroxysmal nocturnal hemoglobinuria. *Blood*. 2021;137(10):1304–1309. doi:10.1182/blood.2019003812
19. Risitano AM, Marotta S, Ricci P, et al. Anti-complement treatment for paroxysmal nocturnal hemoglobinuria: time for proximal complement inhibition? A position paper from the SAAWP of the EBMT. *Front Immunol*. 2019;10:1157. doi:10.3389/fimmu.2019.01157
20. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. *Blood*. 2009;113(17):4094–4100. doi:10.1182/blood-2008-11-189944
21. Brodsky RA, Peffault de Latour R, Rottinghaus ST, et al. Characterization of breakthrough hemolysis events observed in the phase 3 randomized studies of ravulizumab versus eculizumab in adults with paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2021;106(1):230–237. doi:10.3324/haematol.2019.236877
22. Harder MJ, Kuhn N, Schrezenmeier H, et al. Incomplete inhibition by eculizumab: mechanistic evidence for residual C5 activity during strong complement activation. *Blood*. 2017;129(8):970–980. doi:10.1182/blood-2016-08-732800
23. Miyasaka N, Miura O, Kawaguchi T, et al. Pregnancy outcomes of patients with paroxysmal nocturnal hemoglobinuria treated with eculizumab: a Japanese experience and updated review. *Int J Hematol*. 2016;103(6):703–712. doi:10.1007/s12185-016-1946-x
24. Kelly RJ, Hochsmann B, Szer J, et al. Eculizumab in pregnant patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2015;373(11):1032–1039. doi:10.1056/NEJMoa1502950
25. Nakayama H, Usuki K, Echizen H, Ogawa R, Orii T. Eculizumab dosing intervals longer than 17 days may be associated with greater risk of breakthrough hemolysis in patients with paroxysmal nocturnal hemoglobinuria. *Biol Pharm Bull*. 2016;39(2):285–288. doi:10.1248/bpb.b15-00703
26. McKinley CE, Richards SJ, Munir T, et al. Extravascular hemolysis due to C3-loading in patients with PNH treated with eculizumab: defining the clinical syndrome. *Blood*. 2017;130(1):3471.
27. Hill A, Rother RP, Arnold L, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. *Haematologica*. 2010;95(4):567–573. doi:10.3324/haematol.2009.007229
28. Rondelli T, Risitano AM, Peffault de Latour R, et al. Polymorphism of the complement receptor 1 gene correlates with the hematologic response to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2014;99(2):262–266. doi:10.3324/haematol.2013.090001
29. Prata PH, Galimard JE, Sicre de Fontbrune F, et al. Rare germline complement factor H variants in patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2023;141(15):1812–1816. doi:10.1182/blood.2022017019
30. Nishimura J, Yamamoto M, Hayashi S, et al. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med*. 2014;370(7):632–639. doi:10.1056/NEJMoa1311084
31. Socie G, Caby-Tosi MP, Marantz JL, et al. Eculizumab in paroxysmal nocturnal haemoglobinuria and atypical haemolytic uraemic syndrome: 10-year pharmacovigilance analysis. *Br J Haematol*. 2019;185(2):297–310. doi:10.1111/bjh.15790
32. McNamara LA, Topaz N, Wang X, Hariri S, Fox L, MacNeil JR. High risk for invasive meningococcal disease among patients receiving Eculizumab (Soliris) despite receipt of meningococcal vaccine. *MMWR Morb Mortal Wkly Rep*. 2017;66(27):734–737. doi:10.15585/mmwr.mm6627e1
33. Crew PE, Abara WE, McCulley L, et al. Disseminated gonococcal infections in patients receiving eculizumab: a case series. *Clin Infect Dis*. 2019;69(4):596–600. doi:10.1093/cid/ciy958
34. Heesterbeek DAC, Angelier ML, Harrison RA, Rooijackers SHM. Complement and bacterial infections: from molecular mechanisms to therapeutic applications. *J Innate Immun*. 2018;10(5–6):455–464. doi:10.1159/000491439
35. Hillmen P, Szer J, Weitz I, et al. Pegcetacoplan versus Eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2021;384(11):1028–1037. doi:10.1056/NEJMoa2029073

36. Yenerel MN, Sicre de Fontbrune F, Piatek C, et al. Phase 3 study of subcutaneous versus intravenous ravulizumab in eculizumab-experienced adult patients with PNH: primary analysis and 1-year follow-up. *Adv Ther.* 2023;40(1):211–232. doi:10.1007/s12325-022-02339-3
37. Liu H, Xia L, Weng J, et al. Efficacy and safety of the C5 inhibitor crovalimab in complement inhibitor-naïve patients with PNH (COMMODORE 3): a multicenter, Phase 3, single-arm study. *Am J Hematol.* 2023;98(9):1407–1414. doi:10.1002/ajh.26998
38. Röth A, He G, Brodsky A, et al. The Phase III, randomized commodore 2 trial: results from a multicenter study of crovalimab vs eculizumab in paroxysmal nocturnal hemoglobinuria (pnh) patients naïve to complement inhibitors. presented at: European Hematology Association; 2023; Frankfurt, Germany. Available from: https://s3.eu-central-1.amazonaws.com/m-anage.com.storage.aha/temp/aha23_abstract_bodies/S181.pdf. Accessed December 02, 2023.
39. Scheinberg P, Cle D, Edwards J, et al. PHASE III randomized, multicenter, open-label commodore 1 trial: comparison of crovalimab vs eculizumab in complement inhibitor-experienced patients with Paroxysmal Nocturnal Hemoglobinuria (PNH). presented at: European Hematology Association; 2023. Available from: https://s3.eu-central-1.amazonaws.com/m-anage.com.storage.aha/temp/aha23_abstract_bodies/S183.pdf. Accessed December 02, 2023.
40. Devalaraja-Narashimha K, Huang C, Cao M, et al. Pharmacokinetics and pharmacodynamics of pozelimab alone or in combination with cemdisiran in non-human primates. *PLoS One.* 2022;17(6):e0269749. doi:10.1371/journal.pone.0269749
41. Browett PJ, Kulasekararaj A, Notaro R, et al. Vemircopan (ALXN2050) monotherapy in paroxysmal nocturnal hemoglobinuria: interim data from a Phase 2 open-label proof-of-concept study. *Blood.* 2022;140(2):717–719. doi:10.1182/blood-2022-169301
42. de Latour RPRA, Kulasekararaj A, Scheinberg P, et al. Oral monotherapy with iptacopan, a proximal complement inhibitor of factor B, has superior efficacy to intravenous terminal complement inhibition with standard of care eculizumab or ravulizumab and favorable safety in patients with paroxysmal nocturnal hemoglobinuria and residual anemia: results from the randomized, active-comparator-controlled, open-label, multicenter, phase III apply-PNH Study. *Blood.* 2022;140(2). doi:10.1182/blood-2022-171469
43. Kulasekararaj AG, Lazana I. Paroxysmal nocturnal hemoglobinuria: where are we going. *Am J Hematol.* 2023;98 Suppl 4:S33–S43. doi:10.1002/ajh.26882.
44. Bektas M, Copley-Merriman C, Khan S, Sarda SP, Shammo JM. Paroxysmal nocturnal hemoglobinuria: patient journey and burden of disease. *J Manag Care Spec Pharm.* 2020;26(12–b Suppl):S8–S14. doi:10.18553/jmcp.2020.26.12-b.s8
45. Rizzardo S, Bansback N, Dragojlovic N, et al. Evaluating Canadians' Values for Drug Coverage Decision Making. *Value Health.* 2019;22(3):362–369. doi:10.1016/j.jval.2018.08.008
46. Lancet Haematology T. The rising cost of orphan drugs. *Lancet Haematol.* 2015;2(11):e456. doi:10.1016/S2352-3026(15)00229-X
47. Patriquin CJ, Kuo KHM. Eculizumab and beyond: the past, present, and future of complement therapeutics. *Transfus Med Rev.* 2019;33(4):256–265. doi:10.1016/j.tmr.2019.09.004
48. Kulagin AD, Ptushkin VV, Lukina EA, et al. Randomized multicenter noninferiority phase III clinical trial of the first biosimilar of eculizumab. *Ann Hematol.* 2021;100(11):2689–2698. doi:10.1007/s00277-021-04624-7
49. Jang JH, Gomez RD, Bumbea H, et al. A phase III, randomised, double-blind, multi-national clinical trial comparing SB12 (proposed eculizumab biosimilar) and reference eculizumab in patients with paroxysmal nocturnal haemoglobinuria. *EJHaem.* 2023;4(1):26–36. doi:10.1002/jha2.632
50. Wong RSM, Navarro-Cabrera JR, Comia NS, et al. Pegcetacoplan controls hemolysis in complement inhibitor-naïve patients with paroxysmal nocturnal hemoglobinuria. *Blood Adv.* 2023;7(11):2468–2478. doi:10.1182/bloodadvances.2022009129
51. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2004;350(6):552–559. doi:10.1056/NEJMoa031688
52. Kulasekararaj AG, Brodsky RA, Nishimura JI, Patriquin CJ, Schrezenmeier H. The importance of terminal complement inhibition in paroxysmal nocturnal hemoglobinuria. *Ther Adv Hematol.* 2022;13:20406207221091046. doi:10.1177/20406207221091046
53. Debureaux PE, Kulasekararaj AG, Cacace F, et al. Categorizing hematological response to eculizumab in paroxysmal nocturnal hemoglobinuria: a multicenter real-life study. *Bone Marrow Transplant.* 2021;56(10):2600–2602. doi:10.1038/s41409-021-01372-0
54. Kelly RJ, Hill A, Arnold LM, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood.* 2011;117(25):6786–6792. doi:10.1182/blood-2011-02-333997
55. Loschi M, Porcher R, Barraco F, et al. Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: a treatment versus no-treatment study. *Am J Hematol.* 2016;91(4):366–370. doi:10.1002/ajh.24278
56. Hillmen P, Muus P, Duhrsen U, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood.* 2007;110(12):4123–4128. doi:10.1182/blood-2007-06-095646
57. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2006;355(12):1233–1243. doi:10.1056/NEJMoa061648
58. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood.* 2008;111(4):1840–1847. doi:10.1182/blood-2007-06-094136
59. Peffault de Latour R, Brodsky RA, Ortiz S, et al. Pharmacokinetic and pharmacodynamic effects of ravulizumab and eculizumab on complement component 5 in adults with paroxysmal nocturnal haemoglobinuria: results of two phase 3 randomised, multicentre studies. *Br J Haematol.* 2020;191(3):476–485. doi:10.1111/bjh.16711
60. Kulasekararaj A, Brodsky R, Griffin M, et al. Long-term complement inhibition and survival outcomes in patients with paroxysmal nocturnal hemoglobinuria: an interim analysis of the ravulizumab clinical trials. *Hemasphere.* 2022;6:706–707. doi:10.1097/01.HS9.0000846132.33920.32
61. Roth A, Nishimura JI, Nagy Z, et al. The complement C5 inhibitor crovalimab in paroxysmal nocturnal hemoglobinuria. *Blood.* 2020;135(12):912–920. doi:10.1182/blood.2019003399
62. Roth A, Ichikawa S, Ito Y, et al. Crovalimab treatment in patients with paroxysmal nocturnal haemoglobinuria: long-term results from the phase I/II COMPOSER trial. *Eur J Haematol.* 2023;111(2):300–310. doi:10.1111/ejh.14011
63. Latuszek A, Liu Y, Olsen O, et al. Inhibition of complement pathway activation with Pozelimab, a fully human antibody to complement component C5. *PLoS One.* 2020;15(5):e0231892. doi:10.1371/journal.pone.0231892
64. Badri P, Jiang X, Borodovsky A, et al. Pharmacokinetic and pharmacodynamic properties of cemdisiran, an RNAi therapeutic targeting complement component 5, in healthy subjects and patients with paroxysmal nocturnal hemoglobinuria. *Clin Pharmacokinet.* 2021;60(3):365–378. doi:10.1007/s40262-020-00940-9

65. Nishimura JI, Ando K, Masuko M, et al. Tesidolumab (LFG316) for treatment of C5-variant patients with paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2022;107(6):1483–1488. doi:10.3324/haematol.2020.265868
66. Kulasekararaj A, Brodsky R, Kulagin A, Jang JH. Biosimilars in rare diseases: a focus on paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2023;108(5):1232–1243. doi:10.3324/haematol.2022.281562
67. Kulasekararaj A, Lanza F, Arvanitakis A, et al. Efficacy and safety of biosimilar candidate ABP 959 as compared with eculizumab reference product in paroxysmal nocturnal hemoglobinuria. *Blood*. 2022;140(1):8660–8662. doi:10.1182/blood-2022-166722
68. Chow V, Pan J, Chien D, Mytych DT, Hanes V. A randomized, double-blind, single-dose, three-arm, parallel group study to determine pharmacokinetic similarity of ABP 959 and eculizumab (Soliris(R)) in healthy male subjects. *Eur J Haematol*. 2020;105(1):66–74. doi:10.1111/ejh.13411
69. Lee HA, Jang H, Jeong D, Kim Y, Fuhr R. A randomized, double-blind, three-arm, parallel group, single-dose Phase I study to evaluate the pharmacokinetic similarity between SB12 (a proposed eculizumab biosimilar) and eculizumab (Soliris) in healthy subjects. *Int J Clin Pharmacol Ther*. 2022;60(6):269–279. doi:10.5414/CP204176
70. Gusarova V, Degterev M, Lyagoskin I, et al. Analytical and functional similarity of biosimilar Elizaria(R) with eculizumab reference product. *J Pharm Biomed Anal*. 2022;220:115004. doi:10.1016/j.jpba.2022.115004
71. Mastellos DC, Yancopoulos D, Kokkinos P, et al. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. *Eur J Clin Invest*. 2015;45(4):423–440. doi:10.1111/eci.12419
72. Lee JW, Brodsky RA, Nishimura JI, Kulasekararaj AG. The role of the alternative pathway in paroxysmal nocturnal hemoglobinuria and emerging treatments. *Expert Rev Clin Pharmacol*. 2022;15(7):851–861. doi:10.1080/17512433.2022.2109462
73. Panse J, Wilson K, Fishman J, et al. Fatigue and health-related quality of life in paroxysmal nocturnal haemoglobinuria: a post hoc analysis of the pegcetacoplan PEGASUS trial data. *Eur J Haematol*. 2023;111(1):72–83. doi:10.1111/ejh.13969
74. Patriquin CJ, Bogdanovic A, Griffin M, et al. Long-Term Safety and Efficacy of Pegcetacoplan Treatment in Adults with Paroxysmal Nocturnal Hemoglobinuria. *Blood*. 2022;140(1):2921–2923. doi:10.1182/blood-2022-163625
75. M G, Kelly R, Deeren D, et al. Intensive pegcetacoplan dosing in the management of acute hemolysis as part of the 307 open-label extension study. presented at: American Society of Hematology; 2022; New Orleans. Available from: <https://ashpublications.org/blood/article/140/Supplement%201/2937/492951/Intensive-Pegcetacoplan-Dosing-in-the-Management>. Accessed December 02, 2023.
76. Griffin M, Muus P, Munir T, et al. Experience of compassionate-use pegcetacoplan for paroxysmal nocturnal hemoglobinuria. *Blood*. 2023;141(1):116–120. doi:10.1182/blood.2022017266
77. Risitano AM, Roth A, Soret J, et al. Addition of iptacopan, an oral factor B inhibitor, to eculizumab in patients with paroxysmal nocturnal haemoglobinuria and active haemolysis: an open-label, single-arm, phase 2, proof-of-concept trial. *Lancet Haematol*. 2021;8(5):e344–e354. doi:10.1016/S2352-3026(21)00028-4
78. Novartis Phase III APPOINT-PNH trial shows investigational oral monotherapy iptacopan improves hemoglobin to near-normal levels, leading to transfusion Independence in all treatment-naïve PNH patients. 2023. Available from: <https://www.novartis.com/news/media-releases/novartis-phase-iii-appoint-pnh-trial-shows-investigational-oral-monotherapy-iptacopan-improves-hemoglobin-near-normal-levels-leading-transfusion-independence-all-treatment-naive-pnh-patients>. Accessed December 02, 2023.
79. Risitano A, Han B, Ueda Y, et al. Oral complement factor B inhibitor iptacopan monotherapy improves hemoglobin to normal/near-normal levels in paroxysmal nocturnal hemoglobinuria patients naïve to complement inhibitors: phase III APPOINT-PNH trial; 2023.
80. Barratt J, Weitz I. Complement factor D as a strategic target for regulating the alternative complement pathway. *Front Immunol*. 2021;12:712572. doi:10.3389/fimmu.2021.712572
81. Kulasekararaj A, Füreder W, McDonald A, et al. Factor D inhibition with oral bcx9930 leads to sustained control of hemolysis and symptoms over 48 weeks in subjects with paroxysmal nocturnal hemoglobinuria inadequately controlled on c5 inhibitors. *Hemasphere*. 2022;6:720–721. doi:10.1097/01.HS9.0000846188.51290.75
82. McDonald A, Malherbe JL, Kulasekararaj A, et al. Factor D inhibition with oral bcx9930 monotherapy leads to sustained control of hemolysis and symptoms over 48 weeks in subjects with paroxysmal nocturnal hemoglobinuria naïve to c5 inhibitors. *Hemasphere*. 2022;6:719–720. doi:10.1097/01.HS9.0000846184.35066.7a
83. BioCryst discontinues development of BCX9930 and shifts focus to potential once-daily, oral factor D inhibitor, BCX10013; 2022. Available from: <https://ir.biocryst.com/news-releases/news-release-details/biocryst-discontinues-development-bcx9930-and-shifts-focus>. Accessed December 02, 2023.
84. Kulasekararaj AG, Risitano AM, Maciejewski JP, et al. Phase 2 study of danicopan in patients with paroxysmal nocturnal hemoglobinuria with an inadequate response to eculizumab. *Blood*. 2021;138(20):1928–1938. doi:10.1182/blood.2021011388
85. Kira pharmaceuticals announces first cohort of patients dosed in phase 2 study of KP104 in paroxysmal nocturnal hemoglobinuria (PNH); 2022. Available from: <https://www.prnewswire.com/news-releases/kira-pharmaceuticals-announces-first-cohort-of-patients-dosed-in-phase-2-study-of-kp104-in-paroxysmal-nocturnal-hemoglobinuria-pnh-301689797.html>. Accessed December 02, 2023.
86. Griffin M, Hillmen P, Munir T, et al. Significant hemolysis is not required for thrombosis in paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2019;104(3):e94–e96. doi:10.3324/haematol.2018.198846
87. Almeida AM, Bedrosian C, Cole A, et al. Clinical benefit of eculizumab in patients with no transfusion history in the International Paroxysmal Nocturnal Haemoglobinuria Registry. *Intern Med J*. 2017;47(9):1026–1034. doi:10.1111/imj.13523
88. CADTH. Ravulizumab; 2022. Available from: <https://www.cadth.ca/ravulizumab-0>. Accessed 04 December 2023.
89. de Castro C, Grossi F, Weitz IC, et al. C3 inhibition with pegcetacoplan in subjects with paroxysmal nocturnal hemoglobinuria treated with eculizumab. *Am J Hematol*. 2020;95(11):1334–1343. doi:10.1002/ajh.25960
90. Brodsky RA, Lee JW, Nishimura JI, Szer J. Lactate dehydrogenase versus haemoglobin: which one is the better marker in paroxysmal nocturnal haemoglobinuria? *Br J Haematol*. 2022;196(2):264–265. doi:10.1111/bjh.17860
91. Lee JW, Jang JH, Kim JS, et al. Clinical signs and symptoms associated with increased risk for thrombosis in patients with paroxysmal nocturnal hemoglobinuria from a Korean Registry. *Int J Hematol*. 2013;97(6):749–757. doi:10.1007/s12185-013-1346-4
92. Risitano AM, Kulasekararaj AG, Lee JW, et al. Danicopan: an oral complement factor D inhibitor for paroxysmal nocturnal hemoglobinuria. *Haematologica*. 2021;106(12):3188–3197. doi:10.3324/haematol.2020.261826
93. Jang JH, Pavani R, Aurand L, et al. A phase 2, randomized trial evaluating the safety and efficacy of pozelimab and cemdisiran in patients with paroxysmal nocturnal hemoglobinuria. Presented at: European Hematology Association; 2023; Frankfurt, Germany. Available from: https://s3.eu-central-1.amazonaws.com/m-anage.com/storage.aha/temp/cha23_abstract_bodies/P782.pdf. Accessed December 02, 2023.

94. Summary Safety Review - SOLIRIS (eculizumab) and BEXSERO (Multicomponent Meningococcal B Vaccine [recombinant, adsorbed]) - Assessing the Potential Risk of Hemolysis and Low Hemoglobin in Patients Treated with Soliris and Vaccinated with Bexsero; 2016.
95. Granoff DM, Kim H, Topaz N, MacNeil J, Wang X, McNamara LA. Differential effects of therapeutic complement inhibitors on serum bactericidal activity against non-groupable meningococcal isolates recovered from patients treated with eculizumab. *Haematologica*. 2019;104(8):e340–e344. doi:10.3324/haematol.2018.209692
96. Konar M, Granoff DM. Eculizumab treatment and impaired opsonophagocytic killing of meningococci by whole blood from immunized adults. *Blood*. 2017;130(7):891–899. doi:10.1182/blood-2017-05-781450
97. Patriquin CJ, Kulasekararaj A, Peffault de Latour R, et al. Prophylactic antibiotic use and risk of meningococcal infections in patients with Paroxysmal Nocturnal Hemoglobinuria (PNH) treated with eculizumab who received meningococcal vaccination: results from the international PNH registry. *Blood*. 2019;134(1):4802. doi:10.1182/blood-2019-127005
98. Jodele S, Dandoy CE, Danziger-Isakov L, et al. Terminal complement blockade after hematopoietic stem cell transplantation is safe without meningococcal vaccination. *Biol Blood Marrow Transplant*. 2016;22(7):1337–1340. doi:10.1016/j.bbmt.2016.03.032
99. Luzzatto L, Hyry HI, Schieppati A, et al. Outrageous prices of orphan drugs: a call for collaboration. *Lancet*. 2018;392(10149):791–794. doi:10.1016/S0140-6736(18)31069-9
100. Kulasekararaj AG, Kuter DJ, Griffin M, Weitz IC, Roth A. Biomarkers and laboratory assessments for monitoring the treatment of patients with paroxysmal nocturnal hemoglobinuria: differences between terminal and proximal complement inhibition. *Blood Rev*. 2023;59:101041. doi:10.1016/j.blre.2023.101041

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Current Status and Challenges in Delivering Comprehensive Care for Patients with Hemophilia

Shosaku Nomura

To cite this article: Shosaku Nomura (2023) Current Status and Challenges in Delivering Comprehensive Care for Patients with Hemophilia, Journal of Blood Medicine, , 629-637, DOI: [10.2147/JBM.S446204](https://doi.org/10.2147/JBM.S446204)

To link to this article: <https://doi.org/10.2147/JBM.S446204>



© 2023 Nomura.



Published online: 15 Dec 2023.



Submit your article to this journal



Article views: 623



View related articles



View Crossmark data



Citing articles: 1 View citing articles

Current Status and Challenges in Delivering Comprehensive Care for Patients with Hemophilia

Shosaku Nomura 

Center of Thrombosis and Hemostasis, Kansai Medical University Medical Center, Moriguchi, Osaka, Japan

Correspondence: Shosaku Nomura, Center of Thrombosis and Hemostasis, Kansai Medical University Medical Center, 10-15 Fumizono-cho, Moriguchi, Osaka, 570-8507, Japan, Tel + 81 6 6992 1001, Fax + 81 6 6992 1066, Email shosaku-n@mbp.ocn.ne.jp

Abstract: The importance of comprehensive care as a treatment strategy for patients with hemophilia is recognized worldwide. Comprehensive care entails addressing full spectrum of medical and psychological aspects impacting both patients and their families. The primary objective of comprehensive care for individuals with hemophilia is to enable them to lead their daily lives just as anyone else would. To achieve this goal, it is necessary to have a positive and collaborative approach across various healthcare disciplines. This extends beyond clinical specialists, encompassing pediatricians, hematologists, orthopedic surgeons, dental and oral surgeons, gynecologists, nurses, physical therapists, clinical psychologists, and other professionals from diverse fields. This review article discusses the current status and challenges associated with comprehensive care for patients with hemophilia. We categorize these challenges as follows: hemophilic arthritis, rehabilitation, oral care, transitioning from pediatric to adult care, addressing carrier issues, and providing psychological care. There is still substantial work to be undertaken in addressing these hurdles and advancing the quality of comprehensive care for hemophilia patients.

Keywords: hemophilia, comprehensive care, collaborative approach, psychological care, carrier issues

Introduction

Hemophilia A and B are congenital bleeding disorders characterized by abnormalities in coagulation factors (F) VIII and IX, resulting in impaired coagulation factor production and a heightened propensity for bleeding.^{1,2} Patients with severe phenotypes produce less than 1% (1 IU/dL) of FVIII or FIX activity, experiencing recurrent musculoskeletal, soft tissue, and intracranial hemorrhages, which can sometimes escalate into life-threatening events.³ Moderately affected individuals exhibit residual coagulation factor levels between 1–5% of normal, and spontaneous bleeding occurrence is rare; however, there is a risk of bleeding due to trauma. Conversely, in patients with mild hemophilia with coagulation factor activity of 6% or more, bleeding is usually limited to trauma. Notably, even those with mild hemophilia face a higher risk of intracranial hemorrhage-related mortality compared to individuals without hemophilia.⁴

At present, the established standard of care for patients with severe hemophilia revolves around maintaining normal FVIII or FIX activity levels factor levels at or above 1%. This objective is achieved through the regular prophylactic administration of factor concentrates from an early age, effectively preventing arthropathy and normalizing life expectancy.^{5,6} Additionally, extended half-life formulations have been developed to reduce the frequency of concentrated dosing.^{7,8} Furthermore, the introduction of innovative therapies, such as emicizumab, has been transformative in the clinical management of hemophilia. This therapy not only improve compliance with prophylaxis but also provides alternative treatments for patients with inhibitors.^{9,10} Finally, progress is being made toward gene therapy.^{11,12} Clinical trials for gene replacement therapy are actively developing.¹³

The significance of comprehensive care as a treatment strategy for patients with hemophilia is recognized worldwide. Comprehensive care extends to the thorough management of all medical and psychological aspects impacting patients and their families.¹⁴ To provide optimal care for patients with hemophilia, it is important to understand and recognize the various factors involved.¹⁵ The evolution of comprehensive clinical management for hemophilia has given rise to the establishment

of hemophilia treatment centers (HTCs).¹⁶ The core team members of the HTC include not only hematologists and clinical specialists, but also nurses, physical therapists, clinical psychologists, and professionals from various fields.^{17,18} This review aims to discuss the current status and challenges encountered in the realm of comprehensive care for hemophilia patients. In preparing the text, we searched for articles published in PubMed over the past 10 years using the keywords “hemophilia”, “comprehensive care”, and “review”, and selected five articles to serve as reference materials.^{19–23}

Establishment of Comprehensive Care and Its Objectives and Methods

The primary aim of comprehensive care is to enable hemophilia patients to lead their daily lives with the same nor, alcy as anyone else. To fulfill the aforementioned objectives of comprehensive care, it is imperative to involve not only clinical specialists such as pediatricians, hematologists, orthopedists, dental and oral surgeons, and gynecologists but also enlist the active participation of nurses, physical therapists, clinical psychologists, and various other professionals from their respective domains (Figure 1). Specifically, the following intensive efforts are needed.

Foster Intra-Hospital Cooperation and Enhance Medical Care

1. Conduct joint evaluations at an early age (Figure 1A–C).
2. Promptly formulate appropriate treatment plans for patients with moderate disease (Figure 1A and B).
3. Conduct joint consultations between pediatrics and hematology, with an eye towards transitions for patients approaching adulthood (Figure 1A).
4. Provide care for carriers of the disease (Figure 1A and D–F).

Train Medical Personnel

1. Advocate the importance of cooperation among medical departments (Figure 1A).

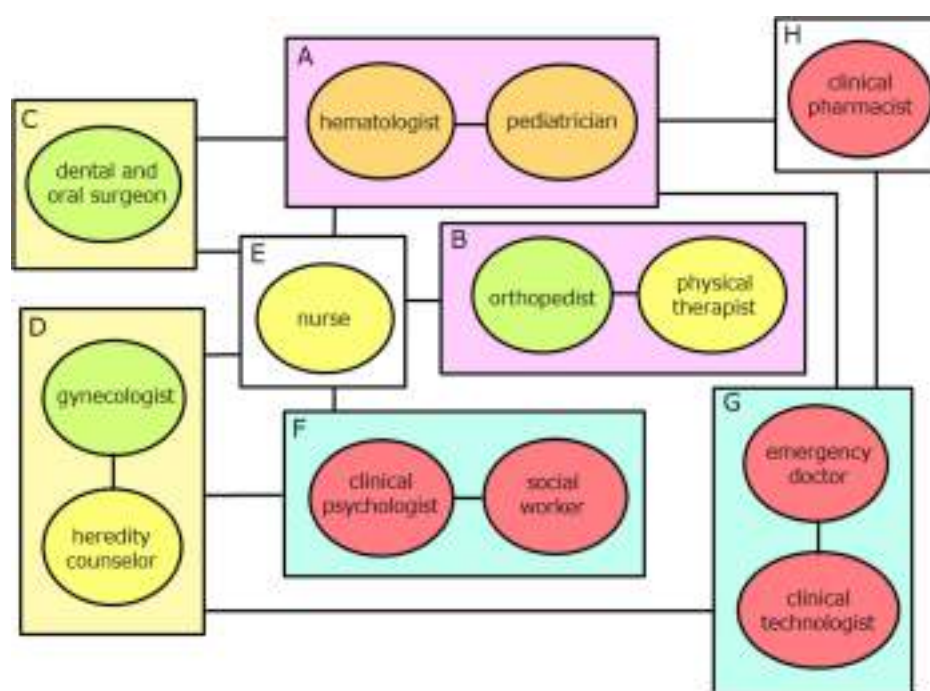


Figure 1 Components of Comprehensive Hemophilia Care. (A) Hematologists and pediatricians play a fundamental role in comprehensive care and complete transitional care; (B) Orthopedist and physical therapist are responsible for joint evaluation and treatment; (C) Dental and oral surgeons are responsible for oral care; (D) Gynecologists and heredity counselors are involved in the care and education of carriers; (E) Nurses are responsible for mental health care as well as assisting in the treatment of each department; (F) Clinical psychologists and social workers are responsible for mental health care as well as supporting social welfare; (G) Emergency doctors and clinical technologist are responsible for the care of patients in the event of an emergency. In addition, the clinical technologist contributes to the medical assistance of each department; (H) Clinical pharmacists provide information on therapeutic drugs to the departments and manage drug inventories.

2. Utilize the center for practical training of students to raise awareness of the disease.
3. Offer specialized training for hemophiliacs in pediatrics and internal medicine, as needed (Figure 1A).

Conduct Public Relations Activities Outside the Hospital

1. Disseminate information regarding the comprehensive care system on the medical institution's website.
2. Extend the scope of comprehensive care to encompass patients in nearby areas.

Hemophilic Arthropathy

Hemophilic arthropathy is caused by repeated intra-articular hemorrhages, with iron deposits in the joints being recognized as the primary cause of this condition.²⁴ Hemophilic arthropathy can manifest in any major joint, such as the shoulder, elbow, hand, hip, knee, and foot, with the elbow, knee, and ankle joints referred to as index joints, being particularly susceptible.^{25,26} Joints that undergo three or more non-traumatic hemorrhages within a six-month period are called target joints.²⁷ In essence, alongside the six index joints, these target joints are extremely important in the evaluation of hemophilic arthropathy.

When a joint experiences bleeding, phagocytic cells in the synovial membrane are triggered into action to remove blood components stored within the joint cavity. This process leads to the development of synovitis.²⁵ According to the guidelines of the World Hemophilia Federation (WHF), chronic synovitis is defined as the persistence of synovitis for over three months.²⁸ MRI studies examining joint changes over time generally reveal that soft tissue changes can be reversed, whereas alterations in bone and cartilage are irreversible.²⁹ Contemporary suggestions recommend the use of refractory synovitis to describe irreversible synovitis that persists for more than three months.³⁰ Therefore, the early diagnosis and appropriate treatment of synovitis are considered important for preventing the progression of arthropathy. Traditionally, the management of hemophilia has centered around patients' complaints of bleeding symptoms. However, relying solely on patient-reported symptoms makes it challenging to identify all instances of bleeding. In the case of micro-hemorrhages without clinical signs, lesions may be identified for the first time through MRI scans in joints exhibiting no subjective symptoms.⁵ Several reports presented in recent years highlight the discrepancy between the patients' self-reported symptoms and the extent of arthropathy.^{31–33} In essence, it has become evident that the diagnosis of hemorrhage and the severity of arthropathy cannot be determined solely based on patient complaints, necessitating an objective test for evaluating hemorrhage and synovitis.

To prevent and manage hemophilic arthropathy effectively, it is crucial to diagnose arthropathy and devise a treatment plan before irreversible bone and cartilage changes occur. As hemorrhage and synovitis changes may manifest as early as childhood, pediatricians must identify joint changes in infants and young children as early as possible. In this context, joint echocardiography is superior to MRI as an imaging evaluation method that simultaneously caters to this target population and purpose, making it particularly valuable for patients with hemophilia.³⁴ In comprehensive care, simplicity and speed are considered key factors in selecting a joint echocardiographic technique for assessing hemophilia arthropathy. One such method well suited for this task is Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US), which has gained popularity.³⁵ Originally designed for outpatient use, this method provides a simple and swift means of detecting early hemophilic arthropathic changes. Research indicates a correlation between the HEAD-US score and the Hemophilia Joint Health Score (HJHS).³⁶ However, some reports have noted limitations in the identification of soft tissue characteristics depicted through arthrography.³⁷ To address this limitation, the Joint Tissue Activity and Damage Examination (JADE) has been newly proposed, allowing the measurement of soft tissue changes and bone and cartilage thickness.³⁸ Accurate recognition of the hemophilic arthropathy status is crucial in determining the course of treatment. While a definitive diagnosis by an orthopedic surgeon remains vital, an initial examination conducted by a pediatrician or internist using joint echocardiography in the routine care setting is also indispensable within the scope of comprehensive care (Figure 1A and B).

Rehabilitation

The most prevalent musculoskeletal observations in patients with hemophilia are blood specimens affecting both muscles and joints.^{39,40} Pediatricians or hematologists are always prepared to address the acute manifestations of hemophilia.^{18,28} Nevertheless, they may not always be adequately equipped to manage the chronic phase of hemophilia and prevent the loss of functional independence. In this context, it is important to underscore the indispensable role that physical therapists play in hemophilia care (Figure 1B).^{41,42}

Treatment and rehabilitation should not be limited solely to patients with established arthropathy but should extend to those encountering unexpected major bleeding events or postoperative musculoskeletal conditions. These scenarios benefit from comprehensive evaluation by a physical therapist working in concert with the hemophiliac and orthopedic surgeon.⁴³ Furthermore, the role of the physical therapist extends to educating both the patient and their parents regarding non-hematologic strategies for addressing bleeding episodes. This includes offering recommendations such as orthotic prescriptions to facilitate the patient's continued physical activity and participation in sports.⁴⁴ Initially, the core objective of physical therapy was to address an individual's exercise requirement and potential.⁴⁵ Notably, guidelines for hemophilia treatment advocate for a diverse array of services, including physical therapy offering specifically tailored for hemophilia patients.^{46,47} Successful treatment of hemophilia requires collaboration between both hemophiliacs and physical therapists. Particularly, the assessment of the musculoskeletal system by a physical therapist can significantly enhance the quality of therapeutic care.⁴⁸

In recent years, remarkable advancement have been made in hemophilia bleeding control, with the achievement of hemostasis now considered a matter of course.^{7,10,49} Moreover, the rapid progression of therapeutic modalities aimed at curing hemophilia, such as gene therapy, is undeniable promising.^{11–13,50,51} These latest advances in hemophilia treatment have had a major impact on the development of comprehensive care strategies. However, the need for appropriate rehabilitation by skilled physical therapists remains (Figure 1B).⁵²

Oral Care

Oral healthcare encompasses a comprehensive approach aimed at preserving the well-being of the oral cavity, its adjacent structures, and associated organs.⁵³ Oral care is extremely important in maintaining fundamental oral functions such as eating, chewing, swallowing, and articulation.⁵⁴ In the context of hemophilia, special measures are required for dental care due to the heightened risk of bleeding.⁵⁵ Many individuals with hemophilia tend to postpone dental visits, even when displaying symptoms of tooth decay or periodontal disease, owing to the fear of triggering a bleeding episode. Oral bleeding incidents in individuals with hemophilia are more prone to occur when they experience falls that impact their mouth or unintentionally bite oral soft tissue.^{55,56} Infants face an additional risk associated with tooth eruption.⁵⁷ Conversely, tooth decay and periodontal disease, if severe and untreated, are not infrequent causes of irreversible bleeding.⁵⁸ Properly maintained gums are less susceptible to bleeding during activities such as brushing. Thus, individuals with hemophilia should become familiar with daily oral care practices.⁵⁹

To provide effective dental care to patients with hemophilia, it is important that they possess a solid awareness of their dental health condition. This, in turn, necessitates instruction in proper brushing techniques and education on effective preventive dental care methods.⁶⁰ A comprehensive hemophilia practice can accomplish these goals (Figure 1A, C and E). Close collaboration among the patient, their family, and healthcare professionals can lead to successful oral healthcare management.⁶¹ It is evident that individuals with hemophilia experience a lower quality of life with respect to oral health compared to their healthy peers.⁵⁹ Therefore, it is strongly advised that individuals with hemophilia receive routine preventive dental care at a hospital or clinic. They should undergo check-ups every six months, and the frequency may need to be increased based on their dental health status. In this context, both visual examination and imaging techniques can be valuable.⁶² Regular oral care is recommended to improve the quality of life for individuals with hemophilia.

Transition

The transition from pediatric to adult care has garnered significant attention across various medical conditions. It is crucial to acknowledge that environmental factors surrounding a disease can significantly differ across various ages and stages of development. Although the exact timing of the transition from childhood to adulthood remains uncertain, adolescence serves as a pivotal indicator.⁶³ Thus, comprehensive hemophilia care should commence in early adolescence with a conscientious approach to developmentally sensitive care transitions in mind.⁶⁴

The challenges encountered during the transition from childhood to adulthood are distinct in many respects. Additionally, concurrent medical issues can often complicate the journey into adulthood. In essence, the elements that define this transition should be recognized as unique to pediatric and adult care (Figure 1A).⁶⁵ The transition of care is a critical period for young individuals with hemophilia, and every effort must be made to ensure it does not lead to a decline in their quality of life (QOL).

Table 1 Items to Be Addressed for Transition

1.	Overview of hemophilia (types of deficient coagulation factors)
2.	Assessment of hemophilia severity (activity level of deficient clotting factor)
3.	Identification of the type and quantity of drug product used
4.	Details regarding lysis operation and the securing of blood vessels for the used drug product
5.	Understanding of trough and peak values
6.	Knowledge of emergency response protocols
7.	Awareness of potential complications and prognosis
8.	Familiarity with genetic considerations (implications for spouses and children)
9.	Developing a sense of spiritual independence from parents
10.	Future prospects for one's own profession

Young adults with hemophilia face unique challenges during the transition to adulthood. These include issues associated with switching healthcare systems, cultivating mature interpersonal relationships, and establishing independent careers. Furthermore, recent years have witnessed the use of safer factor products during childhood, resulting in fewer joint problems and lower rates of HIV transmission, all of which may be significantly different from the experiences of older patients with hemophilia.⁶⁶ To avoid complications following the transition, it is imperative for patients, their families, and healthcare professionals to be fully aware of the fundamental prerequisites for a successful transition from pediatric to adult care (Table 1). It is also imperative to fully recognize the importance of facilitating the transfer of hemophilia patients and establishing suitable transition programs to cater to the needs of the participants.^{64,67}

Carrier

Because of the X-linked inheritance of hemophilia, it is a common belief that the condition primarily affects males, with female carriers assumed to be asymptomatic. However, it is important to note that female carriers also exhibit low coagulation factor activity levels, which can result in abnormal bleeding. In some instances, these cases should be classified as hemophilia.⁶⁸ A previous cross-sectional observational study of female carriers found reduced coagulation factor levels in 23% of eligible cases, confirming an association with bleeding symptoms.⁶⁹ In such cases, invasive procedures should be conducted after optimizing hemostasis, as in patients with mild hemophilia.⁷⁰

Most potential carriers comprise adolescent and young adult women who have yet to experience childbirth and consequently may lack knowledge regarding the risks of being carriers. Therefore, presumptive carriers should be identified through genetic counseling starting around the age of 16.⁷⁰ In the course of counseling, they should receive comprehensive information and active education regarding their reproductive choices, including options such as acceptance of risk, prenatal diagnosis, and preimplantation genetic diagnosis (Figure 1D).⁷¹ In the absence of specific bleeding symptoms, carriers may be unaware of their condition. Thus, participation in comprehensive care can be instrumental in making them aware of their susceptibility to bleeding disorders.

The methods for identifying carriers of hemophilia initially relied on phenotyping based on coagulation tests and have more recently evolved to genetic linkage determinations and next-generation sequencing studies that offer more accurate results.^{72,73} Similarly, methods for prenatal diagnosis of hemophilia have advanced from testing FVIII levels in fetal blood samples at 20–22 weeks of gestation to genetic analysis of fetal DNA extracted from chorionic tissue at 11–14 weeks gestation.⁷³ These advancements may also be crucial in enhancing our understanding of carriers within the context of comprehensive hemophilia care.

Mental Health Care

Individuals with hemophilia and their parents grapple with mental stress and anxieties at various stages of the disease.⁷⁴ One psychological challenge encountered by patients at an early age is the perception of a disparity with healthy peers with regard to exercise. As long as the risk of bleeding is a concern, exercise restrictions are often inevitable.⁷⁵ However, during this period, these concerns can be more pronounced in the parents than in the patients themselves,⁷⁶ raising various anxieties, from immediate situations to the future, which can significantly affect their marital relationships.⁷⁴ Clinical psychologists, social workers, and nurses play a significant role in this regard (Figure 1E and F).

Adolescent individuals with hemophilia share the same concerns as their healthy peers of the same age, such as club activities, romantic relationships, and aspirations for higher education and employment.⁷⁷ Many of these concerns are difficult to discuss with relatives, and a clinical psychologist is expected to come into play. Because of advances in coagulation factor replacement therapy, these patients are often more active than one might expect.⁷⁸ In adolescence, their energy levels surge, accompanied by increased muscular strength, potentially leading to a temporary oversight of their condition, even though it has not been cured. Nevertheless, it is crucial for them to maintain full awareness joint conditions might deteriorate, even without visible swelling, necessitating periodic radiographic assessments.^{31,32} In the transition from childhood to adolescence, it is also important to pass on the essential skill of securing the route of administration of therapeutic preparations (Figure 1A). In adult cases, parental assistance in securing the route should no longer be anticipated, thereby amplifying the role of skilled nurses in providing technical guidance (Figure 1E).

Other

Understanding the characteristics of the drug and the frequency of side effects is important in deciding on a therapeutic regimen for a hemophiliac, and checking the availability of the drug at the facility should not be ignored. Explanation of specific coagulation abnormalities to the carrier and their reliability must also not be forgotten (Figure 1D). Furthermore, there are many important issues to be addressed, such as manuals for emergency transport in the event of a sudden change in a patient, confirmation of the presence or absence of inhibitors, and means of obtaining the drugs used (Figure 1A, H and G). After all, it should be known that collaboration among many specialists is the cornerstone of comprehensive care.

Conclusions

The success of comprehensive hemophilia care hinges on the collaborative team approach adopted by the various healthcare professionals involved in the patient's care. Advances in hemophilia-related treatment methods and the constructive contributions from numerous healthcare professionals have led to significant changes in comprehensive care, compared to over three decades ago. Nonetheless, these advancements have also unveiled new challenges.^{79,80} For example, the availability of innovative therapies must be available for comprehensive care centers to maintain high quality care, and the addition of clinical pharmacists to the comprehensive care model for hemophilia must lead to improved patient outcomes and savings in drug costs. In addition, disparities in the healthcare systems across different countries present a major challenge, and treatments suitable for patients have not necessarily reached all populations. Regarding the management of carriers, current efforts may only be scratching the surface. Thus, much work remains to surmount the challenges confronting comprehensive care.

Acknowledgments

The author thanks Ryosuke Matsuno, Yukihiro Noda, Taichi Omachi, Takashi Yamazoe, Minoru Murata, Fumito Kobayashi, Yuka Kojima, Shunsuke Sawada, Aya Yoshida, Akio Kamiya, Meguru Taguchi, Shun Ito, Reimi Matsuda, Akiko Matsumoto, Chika Sato and Saki Shimada, participating members of the Hemophilia Comprehensive Care at Kansai Medical University.

Disclosure

S. Nomura: Received honoraria for participation in lectures from Chugai, Novo Nordisk, Sanofi, CSL Behring, Takeda, and Japan Blood Products Organization. The author reports no other conflicts of interest in this work.

References

- Toole JJ, Knopf JL, Wozney JM, et al. Molecular cloning of a cDNA encoding human antihemophilic factor. *Nature*. 1984;312(5992):342–347. doi:10.1038/312342a0
- Camerino G, Grzeschik KH, Jaye M, et al. Regional localization on the human X chromosome and polymorphism of the coagulation factor IX gene (hemophilia B locus). *Proc Natl Acad Sci USA*. 1984;81(2):498–502. doi:10.1073/pnas.81.2.498
- Nathwani AC, Tuddenham EG. Epidemiology of coagulation disorders. *Baillieres Clin Haematol*. 1992;5(2):383–439. doi:10.1016/s0950-3536(11)80025-9
- Loomans JJ, Fijnvandraat K. Mortality caused by intracranial bleeding in non-severe hemophilia a patients: reply. *J Thromb Haemost*. 2017;15(8):1710–1711. doi:10.1111/jth.13756
- Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357(6):535–544. doi:10.1056/NEJMoa067659
- Darby SC, Kan SW, Spooner RJ, et al. Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood*. 2007;110(3):815–825. doi:10.1182/blood-2006-10-050435
- Lambert T, Benson G, Dolan G, et al. Practical aspects of extended half-life products for the treatment of hemophilia. *Ther Adv Hematol*. 2018;9(9):295–308. doi:10.1177/2040620718796429
- Nogami K, Shima M, Fukutake K, et al. Correction to: efficacy and safety of full-length pegylated recombinant factor VIII with extended half-life in previously treated patients with hemophilia A: comparison of data between the general and Japanese study populations. *Int J Hematol*. 2018;107(1):123–124. doi:10.1007/s12185-017-2369-z
- Shima M, Hanabusa H, Taki M, et al. Factor VIII-mimetic function of humanized bispecific antibody in hemophilia A. *N. Engl J Med*. 2016;374(21):2044–2053. doi:10.1056/NEJMoa1511769
- Oldenburg J, Levy GG. Efficacy of emicizumab prophylaxis in hemophilia A with inhibitors. *N Engl J Med*. 2017;377(22):2194–2195. doi:10.1056/NEJMc1712683
- Kaminski TW, Ju EM, Gudapati S, et al. Defenestrated endothelium delays liver-directed gene transfer in hemophilia A mice. *Blood Adv*. 2022;6(12):3729–3734. doi:10.1182/bloodadvances.2021006388
- Rasul E, Hallock R, Hellmann M, et al. Gene therapy in hemophilia: a transformational patient experience. *J Patient Exp*. 2023;10:23743735231193573. doi:10.1177/23743735231193573
- Pipe SW, Gonen-Yaacovi G, Segurado OG. Hemophilia A gene therapy: current and next-generation approaches. *Expert Opin Biol Ther*. 2022;22(9):1099–1115. doi:10.1080/14712598.2022.2002842
- Evatt BL, Black C, Batorova A, Street A, Srivastava A. Comprehensive care for haemophilia around the world. *Haemophilia*. 2004;10(Suppl s4):9–13. doi:10.1111/j.1365-2516.2004.01010.x
- Ruiz-Sáez A. Comprehensive care in haemophilia. *Hematology*. 2012;17(sup1):S141–S143. doi:10.1179/102453312X13336169156492
- Bolton-Maggs PHB. Optimal haemophilia care versus the reality. *Br J Haematol*. 2005;132(6):671–682. doi:10.1111/j.1365-2141.2005.05952.x
- Soucie M, Nuss R, Evatt B, et al. Mortality among males with hemophilia: relations with source of medical care. *Blood*. 2000;96(2):437–442.
- Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19(1):e1–47. doi:10.1111/j.1365-2516.2012.02909.x
- Coppola A, Santoro C, Caterine F, et al. Emerging issues on comprehensive hemophilia care: preventing, identifying, and monitoring age-related comorbidities. *Semin Thromb Hemost*. 2013;39(7):794–802. doi:10.1055/s-0033-1354424
- Yeung CHT, Santesso N, Pai M, et al. Care models in the management of haemophilia: a systematic review. *Haemophilia*. 2016;Suppl(3):31–40. doi:10.1111/hae.13000
- Page D. Comprehensive care for hemophilia and other inherited bleeding disorders. *Transfus Apher Sci*. 2019;58(5):565–568. doi:10.1016/j.transci.2019.08.005
- Valentino LA, Baker JR, Butler R, et al. Integrated hemophilia patient care via a national network of care centers in the United States: a model for rare coagulation disorders. *J Blood Med*. 2021;12:897–911. doi:10.2147/JBM.S325031
- Astermark J, Blatný J, Königs C, Hermans C, Jiménez-Yuste V, Hart DP. Considerations for shared decision management in previously untreated patients with hemophilia A or B. *Ther Adv Hematol*. 2023;14:20406207231165857. doi:10.1177/20406207231165857
- Melchiorre D, Manetti M, Matucci-Cerinic M. Pathophysiology of hemophilic arthropathy. *J Clin Med*. 2017;6(7):63. doi:10.3390/jcm6070063
- Gualtierotti R, Solimeno LP, Peyvandi F. Hemophilic arthropathy: current knowledge and future perspectives. *J Thromb Haemost*. 2021;19(9):2112–2121. doi:10.1111/jth.15444
- Zhao L, Yanf H, Li Y, et al. Joint status and related risk factors in patients with severe hemophilia A: a single-center cross-sectional study. *Hematology*. 2022;27(1):80–87. doi:10.1080/16078454.2021.2019892
- Blanchette VS, Key NS, Ljung LR, et al. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. 2014;12(11):1935–1939. doi:10.1111/jth.12672
- Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia, 3rd edition. *Haemophilia*. 2020;26(Suppl. 6):1–158. doi:10.1111/hae.14046
- Stimec J, Dover S, Pullenayegum E, et al. Magnetic resonance imaging in boys with severe hemophilia A: serial and end-of-study findings from the Canadian Hemophilia Primary Prophylaxis Study. *Res Pract Thromb Haemost*. 2021;5(7):e12565. doi:10.1002/rth2.12565
- Mancuso ME, Holstein K, O'Donnell JS, Lobet S, Klamroth R. Synovitis and joint health in patients with haemophilia: statements from a European e-Delphi consensus study. *Haemophilia*. 2023;29(2):619–628. doi:10.1111/hae.14734
- Jackson MD, O'Brien SH, Stanek J, Dunn AL, Kerlin BA. Inpatient health care utilization in children with hemophilia before and after the Joint Outcome Study publication. *J Pediatr Hematol Oncol*. 2019;41(5):e284–289. doi:10.1097/MPH.0000000000001329
- Warren BB, Thornhill D, Stein J, et al. Young adult outcomes of childhood prophylaxis for severe hemophilia A: results of the Joint Outcome Continuation Study. *Blood Adv*. 2020;4(11):2451–2459. doi:10.1182/bloodadvances.2019001311
- van Leeuwen FHP, Timmer MA, de Jong PA, Fischer K, Foppen W. Screening for subclinical synovial proliferation in haemophilia: a systematic review and meta-analysis comparing physical examination and ultrasound. *Haemophilia*. 2023;29(2):445–455. doi:10.1111/hae.14737

34. Gooding R, Thachil J, Alamelu J, Motwani J, Chowdary P. Asymptomatic joint bleeding and joint health in hemophilia: a review of variables, methods, and biomarkers. *J Blood Med*. 2021;12:209–220. doi:10.2147/JBM.S304597
35. Martinoli C, Alberighi ODC, Di Mino G, et al. Development and definition of a simplified scanning procedure and scoring method for Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US). *Thromb Haemost*. 2013;109(6):1170–1179. doi:10.1160/TH12-11-0874
36. Prasetyo M, Monika R, Tulaar A, Prihatono J, Setiawan SI. Correlation between hemophilia early arthropathy detection with ultrasound (HEAD-US) score and hemophilia joint health score (HJHS) in patients with hemophilic arthropathy. *PLoS One*. 2021;16(4):e0248952. doi:10.1371/journal.pone.0248952
37. von Drygalski A, Moore RE, Nguyen S, et al. Advanced hemophilic arthropathy: sensitivity of soft tissue discrimination with musculoskeletal ultrasound. *J Ultrasound Med*. 2018;37(8):1945–1956. doi:10.1002/jum.14541
38. Volland LM, Zhou JY, Barners RFW, et al. Development and reliability of the joint tissue activity and damage examination for quantitation of structural abnormalities by musculoskeletal ultrasound in hemophilic joints. *J Ultrasound Med*. 2019;38(6):1569–1581. doi:10.1002/jum.14846
39. Nacca CR, Harris AP, Tuttle JR. Hemophilic arthropathy. *Orthopedics*. 2017;40:e940–e946. doi:10.3928/01477447-20170619-05
40. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. *Lancet*. 2016;388(10040):187–197. doi:10.1016/S0140-6736(15)01123-X
41. Heijnen L. Rehabilitation and the role of the physical therapist. *Southeast Asian J Trop Med Public Health*. 1993;24(Suppl. 1):26–29.
42. Lobet S, Hermans C, Stephensen D. The emerging clinical and scientific role of the physiotherapist in haemophilia care. *Haemophilia*. 2020;26(4):560–562. doi:10.1111/hae.14096
43. Boccalandro EA, Begnozzi V, Garofalo S, Pasca S, Peyvandi F. The evolution of physiotherapy in the multidisciplinary management of persons with haemophilia (PWH): a scoping review. *Haemophilia*. 2023;29(1):11–20. doi:10.1111/hae.14661
44. Newman JR, Durben N, Baumann K, et al. Physical therapy within US HTCs: a multicentre survey of utilization, practice patterns and pain management approaches. *Haemophilia*. 2022;28(2):343–350. doi:10.1111/hae.14501
45. Chimenti RL, Frey-Law LA, Sluka KA. A mechanism-based approach to physical therapist management of pain. *Phys Ther*. 2018;98(5):302–314. doi:10.1093/ptj/pty030
46. Colvin BT, Astermark J, Fischer K, et al. European principles of haemophilia care. *Haemophilia*. 2008;14(2):361–374. doi:10.1111/j.1365-2516.2007.01625.x
47. De la Corte-Rodriguez H, Rodriguez-Merchan EC, Alvarez-Roman MT, Jiménez-Yuste V. Applying World Health Organization 2020 guidelines on physical activity and sedentary behavior to people with hemophilia. *Expert Rev Hematol*. 2021;14(5):429–436. doi:10.1080/17474086.2021.1924054
48. Mulder K, McCabe E, Strike K, Nilson J. Developing clinical practice guidelines for physiotherapists working with people with inherited bleeding disorders. *Haemophilia*. 2021;27(4):674–682. doi:10.1111/hae.14327
49. Mannucci PM. Hemophilia therapy: the future has begun. *Haematologica*. 2020;105(3):545–553. doi:10.3324/haematol.2019.232132
50. Nathwani AC. Gene therapy for hemophilia. *Hematology Am Soc Hematol Educ Program*. 2019;2019(1):1–8. doi:10.1182/hematology.2019000007
51. Leebeek FWG, Miesbach W. Gene therapy for hemophilia: a review on clinical benefit, limitations, and remaining issues. *Blood*. 2021;138(11):923–931. doi:10.1182/blood.2019003777
52. Stephensen D, de Kleijn P, Matlary RED, et al. Scope of practice of haemophilia physiotherapists: a European survey. *Haemophilia*. 2019;25(3):514–520. doi:10.1111/hae.13727
53. Parker EJ, Jamieson LM, Broughton J, Albino J, Lawrence HP, Roberts-Thomson K. The oral health of indigenous children: a review of four nations. *J Paediatr Child Health*. 2010;46(9):483–486. doi:10.1111/j.1440-1754.2010.01847.x
54. Ship JA, Duffy V, Jones JA, Langmore S. Geriatric oral health and its impact on eating. *J Am Geriatr Soc*. 1996;44(4):456–464. doi:10.1111/j.1532-5415.1996.tb06419.x
55. Zaliuniene R, Peciuniene V, Brukiene V, Aleksejuniene J. Hemophilia and oral health. *Stomatologija*. 2014;16(4):127–131.
56. Kanjani V, Annigeri RG, Hanagavadi S, Manjunath MR. Comparative analysis of oral health and treatment necessities in hemophilia individuals of Davangere population - a case control study. *J Family Med Prim Care*. 2020;9(9):4774–4777. doi:10.4103/jfmpc.jfmpc_413_20
57. Brecher EA, Lewis CW. Infant oral health. *Pediatr Clin North Am*. 2018;65(5):909–921. doi:10.1016/j.pcl.2018.05.016
58. Czajkowska S, Rupa-Matysek J, Gil L, Surdacka A. Assessment of oral health and healthy habits in adult patients with congenital hemophilia. *Eur J Dent*. 2023;17(1):161–172. doi:10.1055/s-0042-1743156
59. Baskirt EA, Zulfikar GAK G, Zulfikar B. Oral and general health-related quality of life among young patients with haemophilia. *Haemophilia*. 2009;15(1):193–198. doi:10.1111/j.1365-2516.2008.01919.x
60. Anderson JA, Brewer A, Creagh D, et al. Guidance on the dental management of patients with haemophilia and congenital bleeding disorders. *Br Dent J*. 2013;215(10):497–504. doi:10.1038/sj.bdj.2013.1097
61. Chaichareon P, Im-Erbsin T. Comprehensive care of hemophilia: role of the dentist. *Southeast Asian J Trop Med Public Health*. 1993;24(Suppl.1):34–36.
62. Masthoff M, Gerwing M, Masthoff M, et al. Dental imaging - a basic guide for the radiologist. *Rofo*. 2019;191(3):192–198. doi:10.1055/a-0636-4129
63. Karki C. Study of young people attending an adolescent friendly centre. *Kathmandu Univ Med J*. 2004;2(4):324–330.
64. Breakey VR, Blanchette VS, Bolton-Maggs PHB. Towards comprehensive care in transition for young people with haemophilia. *Haemophilia*. 2010;16(6):848–857. doi:10.1111/j.1365-2516.2010.02249.x
65. Blum RW, Garell D, Hodgman CH, et al. Transition from child-centered to adult health-care systems for adolescents with chronic conditions. A position paper of the society for adolescent medicine. *J Adolesc Health*. 1993;14(7):570–576. doi:10.1016/1054-139x(93)90143-d
66. Quon D, Reding M, Guelcher C, et al. Unmet needs in the transition to adulthood: 18- to 30-year-old people with hemophilia. *Am J Hematol*. 2015;90(Suppl2):S17–S22. doi:10.1002/ajh.24219
67. Sun J, Zhou X, Hu N. Factor VIII replacement prophylaxis in patients with hemophilia A transitioning to adults: a systematic literature review. *Orphanet J Rare Dis*. 2021;16(1):287. doi:10.1186/s13023-021-01919-w
68. van Galen KPM, d'Oiron R, James P, et al. A new hemophilia carrier nomenclature to define hemophilia in women and girls: communication from the SSC of the ISTH. *J Thromb Haemost*. 2021;19(8):1883–1887. doi:10.1182/bloodadvances.2021006388
69. Plug I, Mauser-Bunschoten EP, Bröcker-Vriends AHJT, et al. Bleeding in carriers of hemophilia. *Blood*. 2006;108(1):52–56. doi:10.1182/blood-2005-09-3879

70. Fijnvandraat K, Cnossen MH, Leebeek FWG, Peters M. Diagnosis and management of haemophilia. *BMJ*. 2012;344:e2707. doi:10.1136/bmj.e2707
71. Lee CA, Chi C, Pavord SR, et al. The obstetric and gynaecological management of women with inherited bleeding disorders—review with guidelines produced by a taskforce of UK haemophilia centre doctors' organization. *Haemophilia*. 2006;12(4):301–336. doi:10.1111/j.1365-2516.2006.01314.x
72. Inaba H, Shinozawa K, Amano K, Fukutake K. Identification of deep intronic individual variants in patients with hemophilia A by next-generation sequencing of the whole factor VIII gene. *Res Pract Thromb Haemost*. 2017;1(2):264–274. doi:10.1002/rth2.12031
73. Dardik R, Janczar S, Lalezari S, et al. Four decades of carrier detection and prenatal diagnosis in hemophilia A: historical overview, state of the art and future directions. *Int J Mol Sci*. 2023;24(14):11846. doi:10.3390/ijms241411846
74. Coppola A, Cerbone AM, Mancuso G, Mansueto MF, Mazzini C, Zanon E. Confronting the psychological burden of haemophilia. *Haemophilia*. 2011;17(1):21–27. doi:10.1111/j.1365-2516.2010.02280.x
75. Broderick CR, Herbeart RD, Latimer J, et al. Association between physical activity and risk of bleeding in children with hemophilia. *JAMA*. 2012;308(14):1452–1459. doi:10.1001/jama.2012.12727
76. van Balen M, O'Mahony B, Cnossen MH, et al. Patient-relevant health outcomes for hemophilia care: development of an international standard outcomes set. *Res Pract Thromb Haemost*. 2021;5(4):e12488. doi:10.1002/rth2.12488
77. Kempton CL, Stout MM, Barry V, et al. Validation of a new instrument to measure disease-related distress among patients with haemophilia. *Haemophilia*. 2021;27(1):60–68. doi:10.1111/hae.14187
78. Hermans C, Noone D, Benson G, et al. Hemophilia treatment in 2021: choosing the "optimal" treatment using an integrative, patient-oriented approach to shared decision-making between patients and clinicians. *Blood Rev*. 2022;52:100890. doi:10.1016/j.blre.2021.100890
79. Page D. Setting the scene: historical overview of challenges and what led to advances in comprehensive care in developed countries, the Canadian experience. *Haemophilia*. 2020;26(Suppl 3):4–5. doi:10.1111/hae.13885
80. Lee D, Le AO, Meganck M, Chamberland S, Pai A. Adding a clinical hemophilia pharmacist to the hemophilia comprehensive care model improves health care-related outcomes and drug-related costs in an integrated health care system. *Perm J*. 2022;26(3):90–93. doi:10.7812/TPP/21.192

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Hyperviscosity Syndrome Induced Bilateral Visual and Auditory Impairment in Therapy Resistant Waldenström Macroglobulinemia with MYD88 and CXCR4 Mutations

Marie M Plante, ErinMarie O Kimbrough, Amit K Agarwal, Liuyan Jiang, Kirk Bourgeois, Greta C Stamper, Michael W Stewart & Han W Tun

To cite this article: Marie M Plante, ErinMarie O Kimbrough, Amit K Agarwal, Liuyan Jiang, Kirk Bourgeois, Greta C Stamper, Michael W Stewart & Han W Tun (2023) Hyperviscosity Syndrome Induced Bilateral Visual and Auditory Impairment in Therapy Resistant Waldenström Macroglobulinemia with MYD88 and CXCR4 Mutations, Journal of Blood Medicine, , 639-648, DOI: [10.2147/JBM.S424072](https://doi.org/10.2147/JBM.S424072)

To link to this article: <https://doi.org/10.2147/JBM.S424072>



© 2023 Plante et al.



Published online: 15 Dec 2023.



Submit your article to this journal



Article views: 165



View related articles



View Crossmark data



Citing articles: 2 View citing articles

Hyperviscosity Syndrome Induced Bilateral Visual and Auditory Impairment in Therapy Resistant Waldenström Macroglobulinemia with MYD88 and CXCR4 Mutations

Marie M Plante¹, ErinMarie O Kimbrough², Amit K Agarwal³, Liuyan Jiang⁴, Kirk Bourgeois⁴, Greta C Stamper⁵, Michael W Stewart⁶, Han W Tun²

¹Department of Internal Medicine, Mayo Clinic, Jacksonville, FL, USA; ²Division of Hematology and Oncology, Mayo Clinic, Jacksonville, FL, USA; ³Department of Radiology, Mayo Clinic, Jacksonville, FL, USA; ⁴Department of Pathology, Mayo Clinic, Jacksonville, FL, USA; ⁵Department of Otolaryngology and Audiology, Mayo Clinic, Jacksonville, FL, USA; ⁶Department of Ophthalmology, Mayo Clinic, Jacksonville, FL, USA

Correspondence: Han W Tun, Division of Hematology and Oncology, Mayo Clinic, 4500 San Pablo Road S, Jacksonville, FL, USA, Tel +1 904 953 7290, Fax +1 904 953 2315, Email Tun.Han@mayo.edu

Abstract: Hyperviscosity syndrome (HVS) is an emergent complication of Waldenström macroglobulinemia (WM) characterized by visual, neurologic, and rarely auditory impairment. We report a 69-year-old female with MYD88 and CXCR4-mutant WM who developed HVS resulting in bilateral blindness and deafness associated with neurologic manifestations including confusion, severe generalized weakness, and imbalance. Ophthalmologic evaluation revealed bilateral central retinal vein occlusion (CRVO), diffuse retinal hemorrhages, macular edema, and serous macular detachments (SMD). Magnetic resonance imaging of the brain showed bleeding in the inner ears. Management was challenging as her WM was resistant to systemic therapies including bendamustine + rituximab (BR) and rituximab + bortezomib + dexamethasone (RVD). Bruton's tyrosine kinase inhibitors could not be used initially due to ongoing lower gastrointestinal bleeding. She required five total sessions of plasma exchange and was finally initiated on zanubrutinib, achieving a partial response. She also received intravitreal bevacizumab with rapid resolution of the retinal hemorrhages but with little improvement of the SMD. She had partial restoration of her hearing in the right ear and only slight improvement in her bilateral visual deficits. The management of HVS in frail, elderly patients with therapy-resistant WM can be challenging. In these cases, plasma exchange is required until an effective systemic therapy can be safely instituted. Genomic profiling is important in the management of WM as it can predict treatment resistance and guide therapeutic decisions.

Keywords: hyperviscosity syndrome, Waldenström macroglobulinemia, central retinal vein occlusion, exudative maculopathy, serous macular detachment, cochlear hemorrhage, plasma exchange, genomic sequencing, CXCR4

Introduction

Waldenström macroglobulinemia (WM) is a lymphoplasmacytic lymphoma, predominantly in the bone marrow, associated with an immunoglobulin M (IgM) monoclonal protein of any size.¹ It is a B-cell non-Hodgkin lymphoma with an estimated incidence of 3–4 cases per million people per year. It is more common among white males with a mean age of 65–70 years at diagnosis.^{2–4} Structurally, IgM molecules are large and form pentamers.⁵ Consequently, the presence of an increased IgM monoclonal protein can give rise to hyperviscosity syndrome (HVS). HVS is a clinical diagnosis aided by laboratory and pathology findings, therefore serum viscosity measurement is not required to make the diagnosis.⁵ Although there is no strict immunoglobulin level or viscosity level threshold to define HVS, there are well-characterized associated ranges that may better clarify true HVS versus an alternative diagnosis.⁵ HVS can develop at different IgM and viscosity levels and vary from patient to patient, however it typically occurs at a viscosity level ≥ 4.0 centipoise (cPoise) and only develops ~15% of WM patients with an IgM level above 6000 mg/dL.^{5–8} HVS occurs in 10–30% of patients with WM, and WM

accounts for nearly 85% of HVS cases.^{9,10} In a cohort of 825 newly diagnosed WM patients, 14% developed symptoms consistent with HVS.¹¹ CXCR4 mutations, found in 30% of patients with WM, are associated with increased likelihood of developing HVS.¹²

The typical clinical manifestations of HVS include visual disturbances—most often blurred vision, mucosal hemorrhage, and neurologic abnormalities.⁶ Less commonly, patients can develop cardiac manifestations such as new onset high-output heart failure or pulmonary hypertension.^{5,6} Other frequent symptoms include bilateral epistaxis or gingival bleeding and visual changes with retinopathy which results from shearing forces that damage small, susceptible vessels.⁶ Exposed venules overlying mucosal surfaces are especially prone to bleeding because of poor underlying support, which occurs in the oropharynx, gingiva, gastrointestinal tract, retina, and on the surface of the brain.⁶ Central neurologic manifestations can range from mild headache and presyncope to a comatose state.⁶ Peripheral neuropathy can occur and is especially common when auto-IgM antibodies are present.⁵ Other symptoms such as hearing loss or vestibular symptoms can occur from stagnant blood within cochlear veins.⁶ Fundoscopic examination is important in the assessment of HVS. Retinopathy can be present even in an individual without visual complaints. Examination can reveal hemorrhages, exudates, microaneurysms, and papilledema.⁵ Central retinal vein occlusion (CRVO) is described as a “blood and thunder” retina with retinal venous engorgement or “sausaging.” Specific retinal findings such as optic disc swelling, dilated veins, as well as dot/blot and flame hemorrhages in all four quadrants should raise concern for CRVO which is an ophthalmologic emergency.¹³ If clinical suspicion is high for HVS and retinopathy is present, laboratory testing to measure serum viscosity should be collected to confirm the diagnosis along with immediate initiation of plasma exchange to prevent further damage followed by systemic therapy.

We report therapeutic challenges in a frail, elderly patient who developed bilateral blindness and deafness related to HVS in the setting of therapy-resistant WM with CXCR4 mutations.

Case Presentation

Six months before coming to our institution, a 69-year-old white female presented to an outside facility with severe anemia. She was diagnosed with a B-cell lymphoma, likely follicular lymphoma, based on a bone marrow biopsy. She initially received two cycles of bendamustine + rituximab (BR) and developed severe pancytopenia and sepsis. She was then treated with five weekly infusions of single agent rituximab (R). She had a rapid decline in her vision followed by hyperacute onset bilateral deafness associated with vertigo, tinnitus, and imbalance. Magnetic resonance imaging (MRI) of the brain and lumbar puncture were unrevealing. Clinical laboratory studies revealed a serum IgM of 4470 mg/dL (37–286 mg/dL) and serum protein electrophoresis with immunofixation (SPEP with IFE) demonstrated an IgM lambda monoclonal protein with an M-spike of 4.9 g/dL. She continued to decline further, developing bilateral pneumonia and was ultimately discharged to hospice. While enrolled in hospice, she had a rapid improvement in her respiratory status as well as strength and decided to pursue additional evaluation.

She presented to our institution. She was quite ill with bilateral visual and auditory impairment, severe lethargy, and confusion requiring immediate hospitalization for further workup.

Her admission labs were significant for anemia with a hemoglobin of 6.2 g/dL (11.6–15.0 g/dL) and thrombocytopenia with a platelet count of $98 \times 10^9/L$ ($157\text{--}450 \times 10^9/L$). The white blood cell count was normal. Her beta-2-microglobulin (B2M) was elevated at 4.02 mcg/mL (1.21–2.70 mcg/mL), C-reactive protein was normal, and LDH was normal. Quantitative immunoglobulins revealed an elevated IgM of 4770 mg/dL (37–286 mg/dL) with decreased IgA of 43 mg/dL (61–356 mg/dL) and a decreased IgG of 78 mg/dL (767–1590 mg/dL). The lambda free light chains (FLC) were elevated at 25.9 mg/dL (0.57–2.63 mg/dL), kappa FLC were low at 0.18 mg/dL (0.33–1.94 mg/dL), and the kappa/lambda FLC ratio was abnormal at 0.0071 (0.26–1.65). SPEP with IFE confirmed a monoclonal IgM lambda with an M-spike of 3.6 g/dL. Serum viscosity was markedly elevated and > 6.6 cpoise (1.4–1.8 centipoises).

MRI brain revealed bilateral small volume internal ear hemorrhage, [Figure 1A–C](#). Computed tomography of the chest, abdomen, and pelvis did not show any lymphadenopathy or organomegaly. Ophthalmologic examination was significant for decreased visual acuity of 20/150 on the right (OD) and 20/100 on the left (OS). Fundoscopic examination showed diffuse intraretinal hemorrhages, mild venous dilation and tortuosity, and serous macular detachments (SMD), [Figure 2A and B](#).

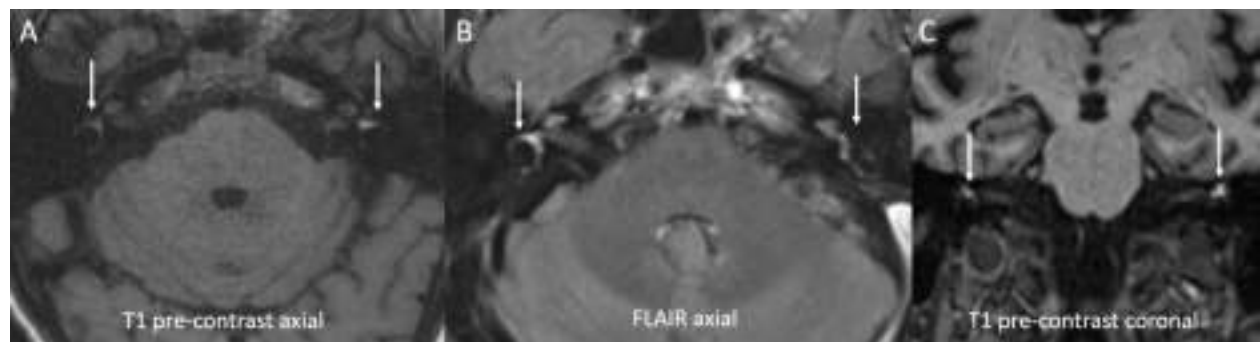


Figure 1 MRI Brain on Initial Presentation. Multiple MRI images reveal bilateral labyrinthine hemorrhages with intrinsic T1 hyperintensity ((**A** and **B**) arrows), more prominent on the left, representing blood products. Loss of normal signal suppression is noted throughout the inner ear on FLAIR image (**C**, arrow).

Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid attenuated inversion recovery.

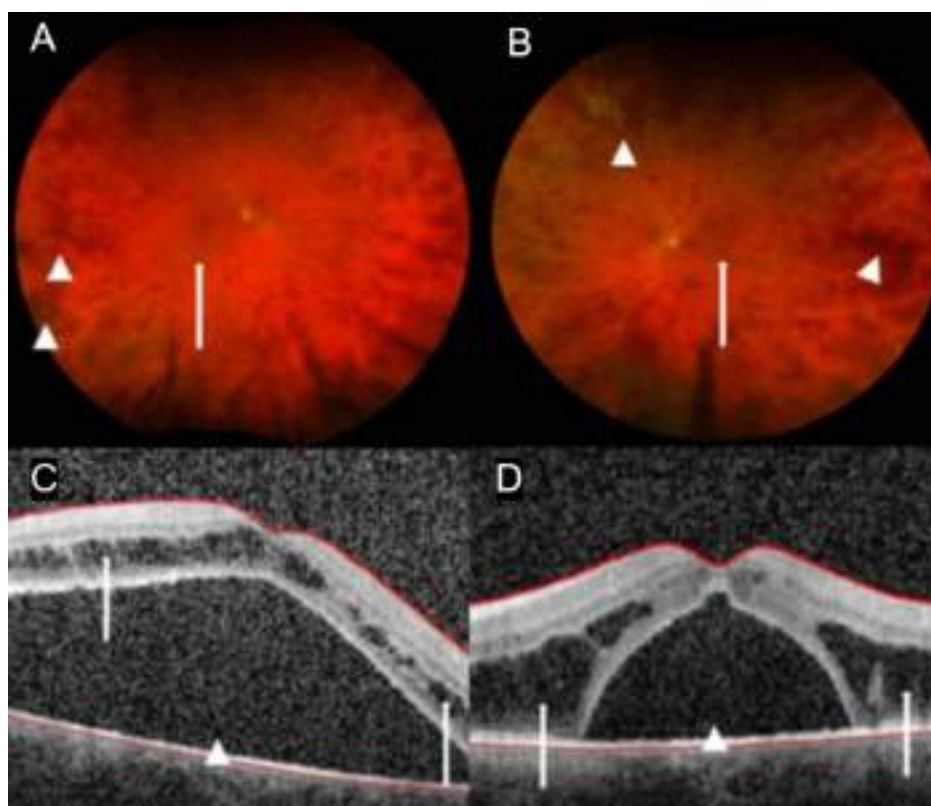


Figure 2 Pre-Treatment Fundoscopic Evaluation and Optical Coherence Tomography. Patient's initial visual acuity of 20/150 OD and 20/100 OS correlates with initial fundoscopic exam showing evidence of venous occlusive disease with circumferential hemorrhages (arrow heads) and macular edema (arrows) on fundus exam ((**A**) OD; (**B**) OS). Initial OCT shows significant bilateral subretinal fluid (arrow heads) and bilateral intraretinal fluid (arrows), more prominent OS ((**C**) OD; (**D**) OS).

Abbreviations: OCT, optical coherence tomography; OD, right eye; OS, left eye; SMD, serous macular detachment; WM, Waldenström macroglobulinemia.

Initial optical coherence tomography (OCT) showed significant subretinal fluid bilateral and intraretinal fluid OS, [Figure 2C](#) and [D](#). These findings were consistent with bilateral CRVO with macular edema and exudative detachments.

Repeat bone marrow biopsy revealed a diffuse and interstitial proliferation of small to medium lymphocytes and plasma cells, [Figure 3A](#). Immunohistochemistry (IHC) studies revealed the lymphoplasmacytic infiltrate was strongly and diffusely positive for CD20, CD79a, and CD138, [Figure 3B–F](#). In addition, light chain stains demonstrated lambda-restriction within the lymphocytes and plasma cells. Flow cytometry identified a monoclonal lambda restricted B-cell population that was negative for CD5, CD10, and CD103. Fluorescence in situ hybridization (FISH) studies were

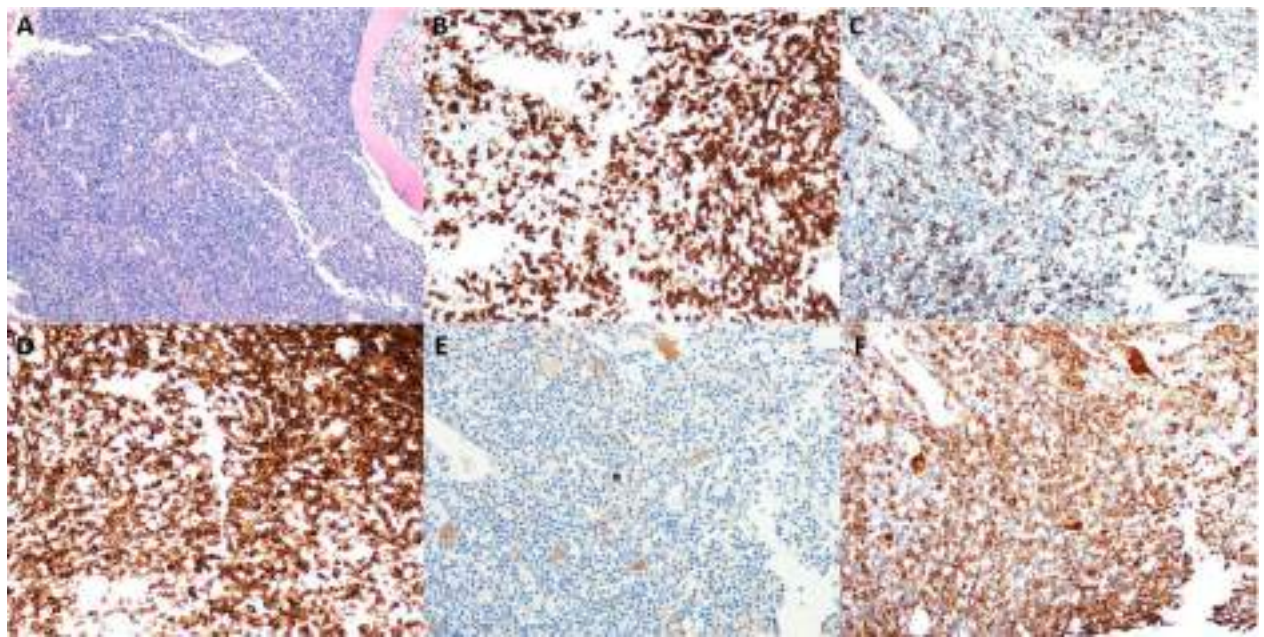


Figure 3 Bone Marrow Biopsy and IHC Staining. Bone marrow biopsy revealed a diffuse and interstitial proliferation of small to medium lymphocytes and plasma cells (A, H&E x 10). IHC studies show that the lymphocytes were strongly and diffusely positive for CD20 (B, x 20); CD138 showed increased plasma cells (C, x 20); and CD79a confirmed the lymphoplasmacytic proliferation by highlighting both neoplastic B-lymphocytes and plasma cells (D, x 20). In addition, the lymphocytes and plasma cells are negative for kappa (E, x 20) but positive for lambda (F, x 20); hence confirmed lambda light chain restriction.
Abbreviations: H&E, hematoxylin and eosin; IHC, immunohistochemistry.

negative for *MYC*, *BCL2*, and *BCL6* gene rearrangements. Molecular studies by polymerase chain reaction (PCR) on the aspiration specimen revealed *MYD88 L265P* and *CXCR4* gene mutations. Next generation sequencing performed on bone marrow aspirate confirmed the *MYD88* and *CXCR4* mutations, and additional pathogenic mutations involving *ETV6*, *ASXL1*, *BCORL1*, and *ATR* were detected. Table 1 provides a summary of the diagnostic evaluation including IHC and

Table 1 Summary of Pathology Results and Genomic Testing

Pathology Findings	
Immunohistochemistry	Positive: CD34, CD138, BCL2, CD79a, CD20, CD3
	Negative: Cyclin D1, BCL6
Light Chain Stains	Lambda+, Kappa-
FISH	Positive: gain of BCL2 gene copy number
	Negative: BCL6, MYC, IGH, and BCL2 gene rearrangements
Flow Cytometry	Lambda restricted B-cells CD19+, CD5-, CD10-, CD103-
Genomic Profile	
Mutation Profile	CXCR4 Ser342PhefsTer6, ETV6 Lys99Glu, ASXL1 Ser156Thr, MYD88 Leu265Pro, BCORL1 Arg1420Ter, ATR Val2265Phe
Mutation Predicted Effect on Protein	Deleterious: ETV6, ASXL1, BCORL1 Tolerated: ATR
Chromosomal Analysis	+4, proximal 17q-, distal 17q+, 18q+
Overexpression	B-cell markers, BCL2 mRNA, BCMA mRNA

genomic testing. Clinical, laboratory, bone marrow pathology, and imaging findings were consistent with HVS secondary to WM. She was immediately started on plasma exchange.

The patient underwent a total of 3 plasma exchange sessions with serial improvement in the IgM levels as follows: 4770 mg/dL, 3550 mg/dL, 2040 mg/dL to 1010 mg/dL on the day of discharge. Unfortunately, her hospitalization was complicated by an episode of significant hematochezia which was attributed to benign rectal and perianal ulcers found on endoscopic evaluation. Due to the GI bleed, retinal hemorrhages, and inner ear hemorrhages, rituximab + bortezomib + dexamethasone (RVD) was initiated in lieu of a Bruton's tyrosine kinase inhibitor (BTKi) to avoid increasing her risk of bleeding. She also received monthly bilateral intravitreal bevacizumab 1.25 mg injections. Her hearing was markedly improved on her right side on the day of discharge. She also had modest improvement in her vision. Her visual acuity improved from 20/100 to 20/50 OS, but there was no improvement OD.

Unfortunately, she had a relapse of the HVS after 2 cycles of RVD. She developed mental status changes and profound weakness in association with a critical rise in IgM to 6680 mg/dL. She was stabilized with two sessions of plasma exchange with IgM level improving to 1850 mg/dL. She was then started on BTKi with zanubrutinib 160 mg twice daily. She responded well to zanubrutinib achieving a partial response with resolution of the anemia and a reduction in the IgM level to 761 mg/dL. A graph demonstrating pertinent IgM levels throughout her clinical course is shown in Figure 4.

Serial fundoscopic examinations are shown with eventual resolution of the retinal hemorrhages (Figure 5A and B). Repeat OCT revealed minimal improvement in the bilateral subretinal fluid collections and intraretinal fluid OS despite intravitreal injections and systemic therapy (Figure 5C and D). Fluorescein angiography (FA) revealed no evidence of retinal vascular or pigment epithelial leakage to explain the etiology of the intra- and subretinal fluid (Figure 5E and F). These findings suggest that her visual symptoms were likely due to WM-related SMD, a type of exudative retinopathy, rather than bilateral CRVO.

In terms of her auditory function, the initial testing revealed significant hearing loss in the right and profound deafness in the left ear (Figure 6A). Repeat audiology testing two months later revealed partial recovery in the right ear but persistent profound deafness in the left ear (Figure 6B). The modest improvement allowed her to obtain amplification hearing aids for use in the right ear to maximize her remaining auditory function.



Figure 4 Clinical Course as Per IgM Levels. Initial IgM levels were treated with 3 sessions of PLEX followed by initiation of RVD. Patient failed therapy on RVD as seen with spike in IgM levels to 6680 mg/dL requiring stabilization with 2 sessions of PLEX. The patient was then started on zanubrutinib, a BTKi, and was able to achieve a PR.

Abbreviations: BTKi, Bruton's tyrosine kinase inhibitor; IgM, immunoglobulin M; PLEX, plasma exchange; PR, partial response; RVD, rituximab + bortezomib + dexamethasone.

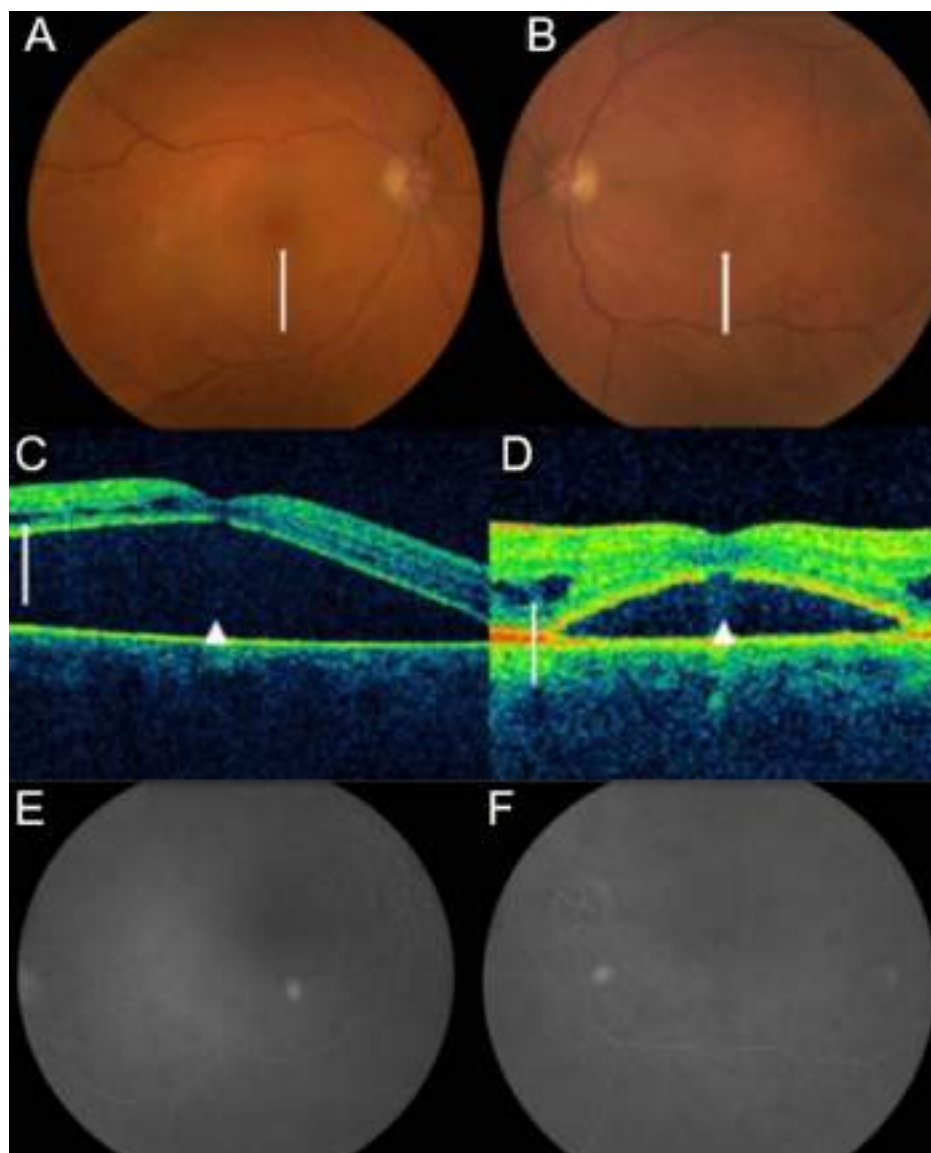


Figure 5 Post-Treatment Fundoscopic Evaluation, Optical Coherence Tomography, and Fluorescein Angiography. **(A)** (OD) and **(B)** (OS) were taken 7 months later and demonstrated significant fundoscopic improvement after plasma exchange, systemic therapy, and intravitreal bevacizumab. Her visual acuity improved to 20/50 OS; however, residual macular edema remains (arrows). After systemic treatment and intravitreal bevacizumab, the intraretinal and subretinal fluid improved but did not resolve on repeat OCT 7 months later and explains only partial visual recovery OS (**(C)** OD; **(D)** OS). These images suggest that the vision loss was primarily due to an exudative retinopathy such as WM-related SMD rather than CRVO as originally diagnosed. Recent FA shows no evidence of vascular leakage to explain the source of intra- and subretinal fluid (**(E)** OD; **(F)** OS).

Abbreviations: CRVO, central retinal vein occlusion; FA, fluorescein angiography; OCT, optical coherence tomography; OD, right eye; OS, left eye; SMD, serous macular detachment; WM, Waldenström macroglobulinemia.

At the time of writing, she remains well and has not required any further plasma exchange. She enjoys a decent quality of life and functional status and is able communicate well. She remains on continuous therapy with zanubrutinib and has not developed any major toxicities.

Discussion

Our case clearly identifies therapeutic challenges in managing frail, elderly patients with therapy-resistant WM-associated HVS. Our patient was likely predisposed to symptomatic HVS based on the CXCR4 mutations as demonstrated in prior studies.¹² Additionally, CXCR4 mutations have been associated with therapy resistance.¹² She was

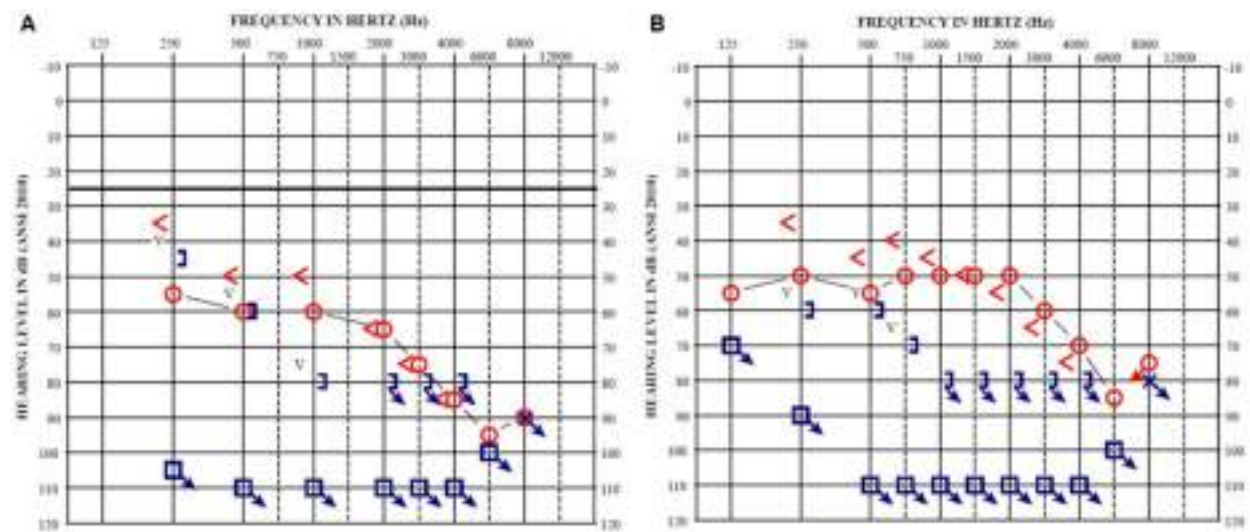


Figure 6 Initial and Post-Treatment Audiogram. **(A)** shows initial audiogram testing; red signifies right ear and blue the left ear. Word recognition was 0% when presented at 100 dB HL in the right ear and could not be tested in the left ear due to a lack of response. Right PTA was 62 dB HL, left ear could not be calculated. The right ear showed a moderate sloping to profound sensorineural hearing loss with very poor word understanding (0%). The left ear did not have any response to sound at the limits of testing. **(B)** shows 2-month repeat audiogram testing after plasma exchange and induction therapy. Word recognition was 53% when presented at 75 dB HL in the right ear and could not be tested in the left ear due to a lack of response. Right PTA was 52 dB HL, left ear could not be calculated. The right ear showed a moderate sloping to severe sensorineural hearing loss with poor word understanding ability (53%). The left ear did not have any response to sound at the limits of testing. The right ear improved an average of 10 dB PTA and showed partial recovery in word understanding ability. This significant improvement resulted in the patient being able to use amplification (hearing aids) successfully. The patient obtained BiCROS hearing aids to maximize her remaining auditory function.

Abbreviations: BiCROS, bi-contralateral routing of sound; dB HL, decibels hearing level; PTA, pure tone average.

resistant to BR and RVD and required multiple sessions of plasma exchange until she responded to zanubrutinib. It is also possible that the development and relapse of HVS was partly due to IgM flare related to rituximab-based therapy together with intrinsic therapy resistance associated with the CXCR4 mutations. Our case represents one of only a few reported cases with concurrent auditory and visual deficits secondary to WM-associated HVS. Furthermore, our patient had SMD, a rare ophthalmologic manifestation of WM with only a few published cases to date.^{14,15} Table 2 summarizes other reported cases of combined auditory and visual deficits in patients with HVS-related WM.

Table 2 Summary of Reported WM-Related HVS with Combined Ophthalmologic and Auditory Manifestations

References	Our Case	Watson et al ¹⁶	Ershler ¹⁷	Sedhom ¹⁸
Age	69	51	57	71
Sex	Female	Male	Male	Male
Auditory Manifestations	Bilateral hearing loss; left > right subjectively	Subjective hearing loss	Subjective hearing loss	Subjective hearing loss
Optical Manifestations				
Symptoms	Blurry vision	Blurry vision	Blurry vision	Floater
Visual acuity (VA)	20/150 OD; 20/100 OS	20/200 OD; 20/40 OS	No other details provided	No other details provided
Fundus exam	Macular edema, retinal hemorrhages	Exudates retinal hemorrhages, vascular dilation, and tortuosity		
OCT	Macular detachment; subretinal and intraretinal fluid bilaterally	Macular detachment; intraretinal fluid bilaterally		
Angiography	Normal	Normal		
IgM Level	4770 mg/dL	n/a	n/a	8090 mg/dL

(Continued)

Table 2 (Continued).

References	Our Case	Watson et al ¹⁶	Ershler ¹⁷	Sedhom ¹⁸
Serum viscosity	6.6 cpoise	n/a	4.2 cpoise	9.2 cpoise
Genetic Mutations	MYD88 CXCR4	n/a	n/a	n/a
Plasma exchange	Yes, x 5 total	Yes	n/a	Yes
Systemic Therapy	BR, RVD, Zanubrutinib, Intravitreal bevacizumab	Bendamustine, Intravitreal bevacizumab	n/a	n/a
Therapeutic Outcomes				
Ophthalmologic Outcomes	VA 20/150 OD & 20/50 OS, resolution of retinal hemorrhages, OCT showed persistent and minimally improved subretinal and intraretinal fluid bilaterally	VA 20/200 OD & 20/30 OS, decreased retinal hemorrhages, OCT showed stable subretinal fluid OD and resolution of subretinal fluid in OS	n/a	n/a
Auditory Outcomes	Sustained left sided hearing loss. Recovery in right ear by an average of 10 dB PTA and partial recovery in word understanding ability. Successful use of hearing aids to maximize right sided hearing.	n/a	n/a	n/a

Abbreviations: BR, bendamustine + rituximab; OD, right eye; OS, left eye; PTA, pure tone average; RVD, rituximab + bortezomib + dexamethasone; OCT, optical coherence tomography; VA, visual acuity.

The visual and auditory manifestations in our patient are quite interesting. The improvement in her hearing was sub-optimal, and she had no significant improvement in her vision. Her auditory impairment was secondary to bleeding within the inner ears and hyperviscosity-induced cochlear damage. She had a partial restoration of her hearing in the right ear with plasma exchange. Her visual impairment did not respond to plasma exchange and intravitreal bevacizumab. Although the hyperviscosity-induced vasculopathy with retinal hemorrhages improved and eventually cleared with therapy, the WM-associated SMD did not improve as evidenced by the persistent intra- and subretinal fluid. The pathophysiology of WM-related SMD has not been fully characterized. Some authors speculate IgM leaks into the subretinal space through damaged external limiting membrane resulting in an osmotic gradient which traps fluid.^{14,15} Others argue the hyperglobulinemia disturbs the retinal pigment epithelium pump and results in an accumulation of subretinal fluid.^{14,15} Based on the findings in our case, we suggest that WM-associated SMD does not respond well to currently available treatments including plasma exchange and intravitreal injection of bevacizumab. Further research is necessary to determine the optimal management of WM-associated SMD.

HVS is an emergent complication of WM and warrants rapid initiation of plasma exchange.⁸ Plasma exchange has been used successfully in the management of HVS since the late 1950s with brisk reversal of retinopathy and other manifestations. It is especially effective in WM as IgM is 80% intravascular. Each session reduces viscosity by 20–30% with a reduction in IgM levels by 30–50%.^{5,8} In a small study of 9 patients with HVS secondary to WM, retinal venous diameter decreased in each patient by an average of 15.3% with a 46.5% average reduction in IgM levels and a 44.7% average reduction in serum viscosity.¹⁹ Plasma exchange requires central line access. A single session lasts approximately 90 minutes with an average of 1–1.5 plasma volumes exchanged per session.^{5,6} It can be repeated on successive days while monitoring serial serum viscosity levels. It is usually not necessary to achieve normal viscosity levels for symptomatic improvement. Plasma exchange should be continued to maintain the serum viscosity below each individual's symptomatic threshold and until systemic pharmacologic therapies take effect.⁵ Our patient required two additional rounds of plasma exchange before she responded to zanubrutinib therapy.

Plasma exchange does not treat the underlying malignant process responsible for HVS; therefore, WM-directed systemic therapy should also be initiated quickly. Under the current NCCN guidelines, WM can be treated with chemoimmunotherapy such as BR, proteasome inhibitor-based therapy such as RVD, and targeted therapy with BTK inhibitors like ibrutinib with or without rituximab.²⁰ As demonstrated in our case, rituximab monotherapy or rituximab-based regimens should be used with caution in the setting of HVS. Rituximab can cause a transient spike in IgM levels in 30–70% of patients. Close monitoring is warranted after initiation of R-based therapies.⁵ Therapy resistance associated

with CXCR4 mutations can complicate the clinical course of WM-associated HVS. Plasma exchange should be repeated for symptomatic patients until significant response to systemic therapy has been achieved.

Genomic profiling of WM can help with predict therapeutic response and guide selection of appropriate systemic therapy.²¹ The most common somatic mutations in WM are MYD88 (95–97%), CXCR4 (30–40%), ARID1A (17%), and CD79B (8–15%).²¹ Our patient had MYD88 and CXCR4 mutations with additional pathogenic mutations involving ETV6, ASXL1, BCORL1, and ATR. The identification and characterization of MYD88 and CXCR4 mutations have encouraged targeted drug development such as BTKi and CXCR4 inhibitors.²¹ ETV6 mutations are present in 3.64% while ATR is altered in 1.54% of non-hodgkin lymphoma patients.²² CXCR4 mutations have been associated with resistance to ibrutinib and varying levels of resistance to bendamustine and bortezomib.⁷ With regards to our patient, she did not respond to bendamustine and bortezomib, and responded well to zanubrutinib. She stopped requiring plasma exchange following the initiation of zanubrutinib.

Conclusion

Therapeutic management of HVS is quite challenging in frail, elderly patients with therapy-resistant WM related to CXCR4 mutations. HVS is an emergent complication of WM which warrants rapid initiation of plasma exchange. Emergent plasma exchange may need to be repeated until an effective systemic therapy has been identified. R-based regimens should be used with caution in the presence of HVS due to IgM flare. WM-associated SMD does not appear to respond to the currently available therapies and warrants further research. Genomic profiling is important in management of WM as it predicts therapy resistance and guides therapeutic decisions.

Abbreviations

B2M, beta-2-microglobulin; BR, bendamustine + rituximab; BTKi, Bruton's tyrosine kinase inhibitor; CRVO, central retinal vein occlusion; cpoise, centipoise; FA, Fluorescein angiography; FISH, fluorescence in situ hybridization; FLC, free light chains; HVS, hyperviscosity syndrome; IF, immunofixation; IgM, immunoglobulin M; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; OCT, optical coherence tomography; OD, right eye; OS, left eye; PCR, polymerase chain reaction; R, rituximab; RVD, rituximab, bortezomib, dexamethasone; SMD, serous macular detachment; SPEP, serum protein electrophoresis; WM, Waldenström Macroglobulinemia.

Consent for Publication

The study participant has given written informed consent to participate as well as consent to publish her data and images. IRB approval was not required to publish the case details.

Acknowledgment

The abstract of this paper was presented virtually at the International Summit on Hematology and Blood Disorders as a poster presentation with interim findings. The poster's abstract is available online at: <https://magnusconferences.com/hematology-blood/program/scientific-program/2023/hyperviscosity-syndrome-induced-bilateral-visual-and-auditory-impairment-in-cxcr4-mutant-waldenstrom-macroglobulinemia>.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Waldenström JG. Macroglobulinemia—a review. *Haematologica*. 1986;71(6):437–440.
2. Groves FD, Travis LB, Devesa SS, et al. Waldenström's macroglobulinemia: incidence patterns in the United States, 1988–1994. *Cancer*. 1998;82(6):1078–1081. doi:10.1002/(SICI)1097-0142(19980315)82:6<1078::AID-CNCR10>3.0.CO;2-3
3. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375–2390. doi:10.1182/blood-2016-01-643569
4. Varettoni M, Ferrari A, Frustaci AM, et al. Younger patients with Waldenström Macroglobulinemia exhibit low risk profile and excellent outcomes in the era of immunotherapy and targeted therapies. *Am J Hematol*. 2020;95(12):1473–1478. doi:10.1002/ajh.25961

5. Stone MJ, Bogen SA. Role of plasmapheresis in Waldenström's macroglobulinemia. *Clin Lymphoma Myeloma Leuk*. 2013;13(2):238–240. doi:10.1016/j.clml.2013.02.013
6. Gertz MA. Acute hyperviscosity: syndromes and management. *Blood*. 2018;132(13):1379–1385. doi:10.1182/blood-2018-06-846816
7. Gertz MA. Waldenström Macroglobulinemia: tailoring therapy for the individual. *J Clin Oncol*. 2022;40(23):2600–2608. doi:10.1200/JCO.22.00495
8. Schwartz J, Padmanabhan A, Aqui N, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: the Seventh Special Issue. *J Clin Apher*. 2016;31(3):149–162. doi:10.1002/jca.21470
9. Stone MJ, Bogen SA. Evidence-based focused review of management of hyperviscosity syndrome. *Blood*. 2012;119(10):2205–2208. doi:10.1182/blood-2011-04-347690
10. Shrestha S, Poddar E, Bashyal B, et al. Bilateral central retinal vein occlusion as an initial presentation of Waldenström macroglobulinemia: a case report. *J Med Case Rep*. 2023;17(1):59. doi:10.1186/s13256-023-03778-4
11. Gustine JN, Meid K, Dubeau T, et al. Serum IgM level as predictor of symptomatic hyperviscosity in patients with Waldenström macroglobulinemia. *Br J Haematol*. 2017;177(5):717–725. doi:10.1111/bjh.14743
12. Castillo JJ, Moreno DF, Arbelaez MI, et al. CXCR4 mutations affect presentation and outcomes in patients with Waldenström macroglobulinemia: a systematic review. *Expert Rev Hematol*. 2019;12(10):873–881. doi:10.1080/17474086.2019.1649132
13. Kiew S, Ting D. Diagnosis and management of central retinal vein occlusion. *EyeNet Magazine*; 2018.
14. Baker PS, Garg SJ, Fineman MS, et al. Serous macular detachment in Waldenström macroglobulinemia: a report of four cases. *Am J Ophthalmol*. 2013;155(3):448–455. doi:10.1016/j.ajo.2012.09.018
15. Leskov I, Knezevic A, Gill MK. Serous macular detachment associated with Waldenström macroglobulinemia managed with ibrutinib: a case report and new insights into pathogenesis. *Retin Cases Brief Rep*. 2021;15(4):490–494. doi:10.1097/ICB.0000000000000837
16. Watson JA, Olson DJ, Zhang AY. Hyperviscosity retinopathy due to Waldenström macroglobulinemia: a case report and literature review. *J Vitreoretin Dis*. 2021;5(6):520–524. doi:10.1177/2474126420987142
17. Ershler W. Waldenström macroglobulinemia with hyperviscosity: illustrative case series. *Relias Media*; 2012.
18. Sedhom R. View of Waldenström's macroglobulinemia presenting as syncope. *Global J Med Res*. 2016;16(F5):17–18.
19. Menke MN, Feke GT, McMeel JW, Treon SP. Effect of plasmapheresis on hyperviscosity-related retinopathy and retinal hemodynamics in patients with Waldenström's macroglobulinemia. *Invest Ophthalmol Vis Sci*. 2008;49(3):1157–1160. doi:10.1167/iops.07-1254
20. Anderson KC, Alsina M, Bensinger W, et al. Waldenström Macroglobulinemia/Lymphoplasmacytic Lymphoma, version 1.2023. *Natl Compr Canc Netw*. 2022;10(10):1211–1219.
21. Treon SP, Xu L, Guerrero ML, et al. Genomic landscape of Waldenström macroglobulinemia and its impact on treatment strategies. *J Clin Oncol*. 2020;38(11):1198–1208. doi:10.1200/JCO.19.02314
22. André F, Amedos M, Baras AS; AACR Project GENIE Consortium. AACR project GENIE: powering precision medicine through an International Consortium. *Cancer Discov*. 2017;7(8):818–831. doi:10.1158/2159-8290.CD-17-0151

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Real-World Amount of Clotting Factor Concentrates Dispensed and Annual Medical Expenditures for Japanese Patients with Hemophilia B

Katsuyuki Fukutake, Kanae Togo, Linghua Xu, Leona E Markson, José Maria Jimenez Alvir, Ian Winburn & Toshiyuki Karumori

To cite this article: Katsuyuki Fukutake, Kanae Togo, Linghua Xu, Leona E Markson, José Maria Jimenez Alvir, Ian Winburn & Toshiyuki Karumori (2023) Real-World Amount of Clotting Factor Concentrates Dispensed and Annual Medical Expenditures for Japanese Patients with Hemophilia B, Journal of Blood Medicine, , 649-661, DOI: [10.2147/JBM.S418818](https://doi.org/10.2147/JBM.S418818)

To link to this article: <https://doi.org/10.2147/JBM.S418818>



© 2023 Fukutake et al.



Published online: 19 Dec 2023.



Submit your article to this journal



Article views: 139



View related articles



View Crossmark data

Real-World Amount of Clotting Factor Concentrates Dispensed and Annual Medical Expenditures for Japanese Patients with Hemophilia B

Katsuyuki Fukutake^{1,2}, Kanae Togo³, Linghua Xu³, Leona E Markson⁴,
José Maria Jimenez Alvir⁵, Ian Winburn⁶, Toshiyuki Karumori³

¹Laboratory Medicine, Tokyo Medical University, Shinjuku, Tokyo, Japan; ²Department of Blood Coagulation Diseases, Ogikubo Hospital, Suginami, Tokyo, Japan; ³Pfizer Japan Inc, Shibuya-ku, Tokyo, Japan; ⁴Pfizer Inc, Collegeville, PA, USA; ⁵Pfizer Inc, New York, NY, USA; ⁶Pfizer Ltd, Surrey, UK

Correspondence: Toshiyuki Karumori, Pfizer Japan Inc, Shinjuku Bunka Quint Bldg, 3-22-7, Yoyogi, Shibuya-ku, Tokyo, Japan, Tel +81-80-4152-4243, Email Toshiyuki.karumori@pfizer.com

Introduction: Until extended half-life (EHL) factor IX (FIX) concentrates became available in Japan in 2010, patients with hemophilia B received intravenous FIX replacement therapy with standard half-life (SHL) FIX concentrates.

Purpose: To investigate the amount of factor dispensed and the associated medical expenditures for the treatment of hemophilia B in the real-world clinical setting in Japan.

Methods: This retrospective study comprised patients with hemophilia B (N=197) who had filled prescriptions for FIX concentrates reported in Japan's Medical Data Vision database from 2015 to 2019. Patients were included if they had 2 or more prescriptions for the same FIX concentrates within the first 6 months of the study period and the interval between prescriptions was at least 2 weeks.

Results: Since 2015, there was a decrease in the proportion of patients using SHL FIX concentrates and a corresponding increase in international units of dispensed EHL FIX concentrates. Median annualized dispensed dosages (IU/kg body weight) of EHL FIX concentrates were lower than for SHL concentrates for outpatient use only. Annual total health care expenditures per patient and annual expenditures for prescribed FIX concentrates increased each year during the study period. Following a switch from an SHL to an EHL concentrate, the median amount of prescribed FIX concentrate decreased slightly, although median total health care expenditures and FIX concentrate expenditures increased.

Conclusion: In the real-world setting in Japan, medical expenditures and the proportion of patients prescribed EHL FIX concentrates for the treatment of hemophilia B have increased.

Keywords: blood coagulation factors, drug labeling, factor IX, health care administrative claims, health care costs

Introduction

Hemophilia B is an inherited clotting disorder caused by missing or defective factor IX (FIX), a serine protease of the coagulation cascade intrinsic pathway.¹ The World Federation of Hemophilia found that in 2021 there were 1252 patients with hemophilia B in Japan, as reported by Japan Foundation for AIDS Prevention.² The main treatment approach for these patients is intravenous FIX replacement therapy.

In Japan, in addition to the plasma-derived and recombinant FIX concentrates with a standard half-life (SHL), several extended half-life (EHL) concentrates have become available since 2010.^{3,4} Eftrenonacog alfa was launched in September 2014, followed by albutrepenonacog alfa in November 2016 and nonacog beta pegol in November 2018.⁵⁻⁷ The SHL concentrate nonacog gamma became available in May 2016. With the development of gene therapy and other novel nonfactor products for management of hemophilia B, it is likely that the treatment paradigm will diversify further.^{8,9}

FIX concentrate dosing and administration regimens vary widely from patient to patient, are often based on the physician's treatment decision, and are governed by the medication's label (ie, whether the medicine is to be used

prophylactically, on demand, for surgery, or for immune tolerance induction).^{3,10} Treatment with FIX concentrates is individually tailored according to the patient's degree of bleeding tendency, level of physical activity, and pharmacokinetics, and therefore varies widely even when used according to the guidelines and label.¹⁰ In addition to hospital-based treatment, home therapy for hemophilia is permitted in Japan and is performed at the patient's discretion.

In patients receiving regular prophylaxis, the use of EHL FIX concentrates can facilitate longer dosing intervals (up to 21 days) versus SHL FIX concentrates (single-dose administration every 3 days or twice per week in Japan).^{10–14} However, expenditures for the same dosage (in international units) of an EHL concentrate may exceed the expenditures for an SHL concentrate.¹⁵ Therefore, it is necessary to understand the actual usage of FIX concentrates in clinical practice and their associated expenditures. Given the need for long-term prophylaxis, expenditure is an important consideration in hemophilia care, and several studies have analyzed direct health care expenditures for different treatment regimens in several countries.^{3,16–18} Two analyses of the Truven Health MarketScan US pharmacy claims databases found that switching to an EHL concentrate was associated with increased median factor expenditures during the 12 months following the switch.^{15,19}

Currently, no published evidence is available regarding real-world treatment patterns for the use of FIX concentrates and hemophilia B treatment expenditures in Japan. The current study analyzed real-world data from a Japanese hospital-based administrative database. The main objective was to investigate treatment of hemophilia B, including the amounts of FIX concentrate in international units (IU) that were dispensed and the medical expenditures for patients who had consistent prescription fills of FIX concentrates and were without factor inhibitors. The prevalence of the two major complications of hemophilia, bleeding during hospitalization and joint damage,²⁰ was also analyzed.

Materials and Methods

Ethical Conduct

This study was conducted in accordance with legal and regulatory requirements in Japan, and included anonymized claims data, with no personal patient data. Japanese Ethical Guidelines for Medical and Biological Research Involving Human Subjects do not apply to studies that use anonymized secondary data; consequently, this study was not reviewed by any institutional review board or ethics committee.

Study Design

This was a retrospective study of patients with hemophilia B included in the Medical Data Vision Co., Ltd (MDV) database, a hospital-based database containing inpatient and outpatient hospital data and prescription data collected after a hospital visit, along with health claims and laboratory data. As of May 2020, the MDV was the largest administrative database in Japan, with data from 413 facilities and more than 31 million patients, covering hospitals from all prefectures. The study period included data from January 1, 2015, through December 31, 2019. The index date was defined as the date of the first prescription fill of the FIX concentrate during the study period, with the disease name recorded as hemophilia B. The patients in the study population could have records pertaining to prescriptions for several FIX concentrates that met this inclusion criterion in the patient's medical history. Treatment duration with each FIX concentrate was defined as the period from the first prescription fill of the FIX concentrate to the day before a switch to a different concentrate, or it was censored at the last prescription of the same concentrate if no switch occurred during the study period.

Study Population

Patients who met all of the following criteria were included: (1) evidence of visiting a health care facility in the MDV database during the study period; (2) diagnosis of hemophilia B (ICD-10 code D67, excluding hypofibrinogenemia) during the study period, excluding suspected diagnosis; and (3) ≥ 2 prescriptions of the same FIX concentrate within the first 6 months of the study period and ≥ 2 weeks apart from each other during the study period, excluding during hospitalization. Exclusion criteria included patients who (1) were diagnosed with a related disease name ("congenital hemophilia with

FIX inhibitor”, Japanese claims code 845703) or (2) received bypassing agents (treatment for hemophilia B with inhibitors) after the index date.

Variables

Prescriptions for both SHL and EHL FIX concentrates were included in this study. For hemophilia B, SHL FIX concentrates have half-lives of approximately 18–40 hours.²¹ The half-life of EHL FIX concentrates is extended by up to five times compared with SHL concentrates.²¹ The following SHL concentrates were included: nonacog alfa, nonacog gamma, and plasma-derived FIX. The following EHL concentrates were included: eftrenonacog alfa, albutrepenonacog alfa, and nonacog beta pegol.

Variables included treatment duration, weight, joint damage, bleeding, and hospitalization. Although the treatment duration could be censored, the summary of treatment duration was used for supporting interpretation of the annualized FIX dosage, described below. Weight was calculated based on the statistical average weight of Japanese individuals in the specified age groups.^{22,23} Joint damage and bleeding during hospitalization were evaluated from inpatient records, regardless of whether joint damage or bleeding were the primary cause of hospitalization. A determination of joint damage and/or bleeding was based on diagnostic information in physicians recorded clinical assessments.

Outcome Measures

The percentage of patients prescribed each FIX concentrate and the annualized dosage (factor IU/kg body weight) of each FIX concentrate were analyzed in an outpatient setting. The prevalence of joint damage and bleeding, expenditures for prescribed FIX concentrates, and expenditures for total health care were measured for inpatients and outpatients.

Annualized dosage was based on reported FIX concentrate prescriptions and the statistical average weight of Japanese individuals in the specified age groups. Median annual expenditures for prescribed FIX concentrates and median annual total health care expenditures were summarized in patients receiving treatment with regular replacement therapy. For the calculation of annual expenditures, regular replacement therapy was defined as the use of FIX concentrates that met each of the following criteria: total treatment period of the FIX concentrates of ≥ 1 year; prescription of any FIX concentrate ≥ 2 times on different dates during the year, and no interval > 6 months between prescriptions. Expenditures per FIX concentrate were derived from the Japanese drug price standard.²⁴ Expenditures in USD were calculated using a conversion of 109 yen/USD.²⁵

The subpopulation of patients (both inpatients and outpatients) for whom data were available for ≥ 3 months before and ≥ 3 months after switching from an SHL concentrate to an EHL concentrate (ie, the first occurrence of a switch to an EHL concentrate) and those who filled ≥ 2 prescriptions for an EHL concentrate were included in the analyses for switching. The IU/kg of FIX concentrate dispensed in the 4 quarters before the switch, as well as expenditures for prescribed FIX concentrates and total health care expenditures after the switch, were recorded.

Data Analysis

Data were summarized and analyzed for the overall study period and by year using descriptive statistics (patient and treatment setting characteristics; bleeding during hospitalization). All analyses were conducted for the age subgroups 0–11, 12–19, 20–64, and ≥ 65 years. The prevalence rate of joint damage, estimated with a 95% confidence interval, was calculated as the number of patients with joint damage during the study period divided by the total follow-up duration of all patients (per 100 patient-years). For the switching analysis, summary statistics for annualized FIX concentrate dosage dispensed in the 4 quarters (3 months, 4–6 months, 7–9 months, and 10–12 months) before and after switching were reported. Incomplete quarters were not included. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study Population

A total of 197 patients with hemophilia B (median age, 25 years; range: < 1 –87 years) with ≥ 2 prescriptions filled for FIX concentrates within the first 6 months of the study period at intervals of > 2 weeks and no prescriptions for bypass

products were included in the analysis (Figure 1). Patient age and comorbidities at baseline are presented in Table 1. Of note, at baseline, 41.6% of the patients had viral hepatitis and 38.6% had chronic viral hepatitis C after sustained virologic response by antiviral therapy. The average follow-up duration for each patient was 3.3 years (median [first quartile to third quartile], 34.5 [30.2–57.8] months). Patients visited university hospitals (44.7% of patients), local public hospitals (33.5%), national hospitals (18.3%), and private hospitals (3.6%), in that order. The majority (71.1%) of the hospitals had more than 500 beds.

Percentage of Patients Prescribed Each FIX Concentrate in an Outpatient Setting

The percentage of patients for whom an SHL concentrate (ie, nonacog alfa, nonacog gamma, and plasma-derived FIX) was dispensed showed a continual decrease from 2015 onward, whereas the overall percentage of patients for whom an EHL concentrate (ie, eftrenonacog alfa, albutrepenonacog alfa, and nonacog beta pegol) was dispensed increased (Figure 2). The percentage of patients prescribed the EHL FIX concentrate albutrepenonacog alfa, launched in 2016, increased across all age categories over the analysis period (2015–2019) (Figure 2 and Supplementary Figures S1–S4).

Annualized Dosage for Each FIX Concentrate in an Outpatient Setting

Median annualized dosages (factor IU/kg body weight) of dispensed EHL FIX concentrates were lower (medians ranging from 1507.4 to 2447.4 IU/kg) than median annualized dosages of SHL FIX concentrates (medians ranging from 2092.9 to 3181.4 IU/kg) for outpatient use only (Table 2), both overall and according to age group (Supplementary Table S1). The treatment duration for each FIX concentrate varied.

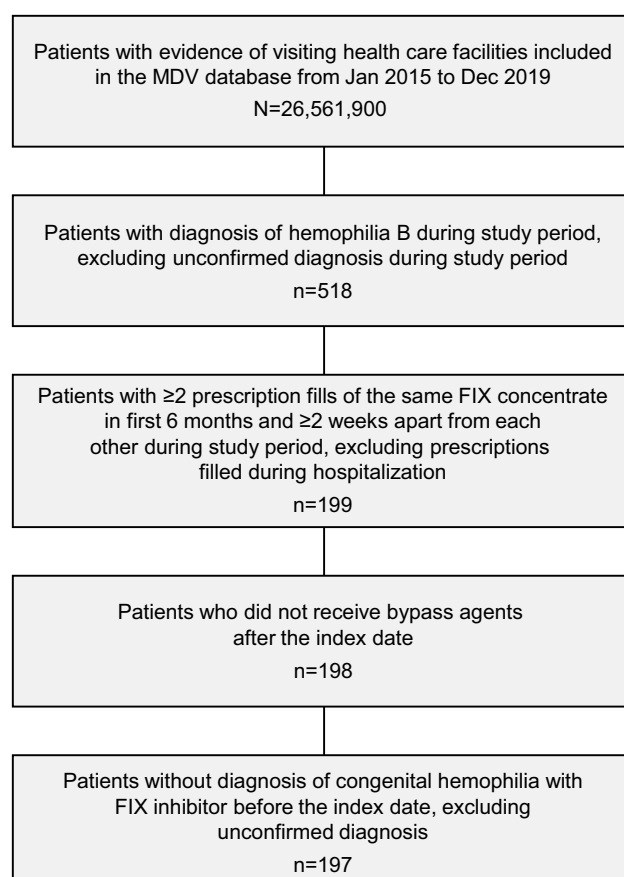


Figure 1 Study cohort selection.

Abbreviations: FIX, factor IX; MDV, Medical Data Vision Co., Ltd.

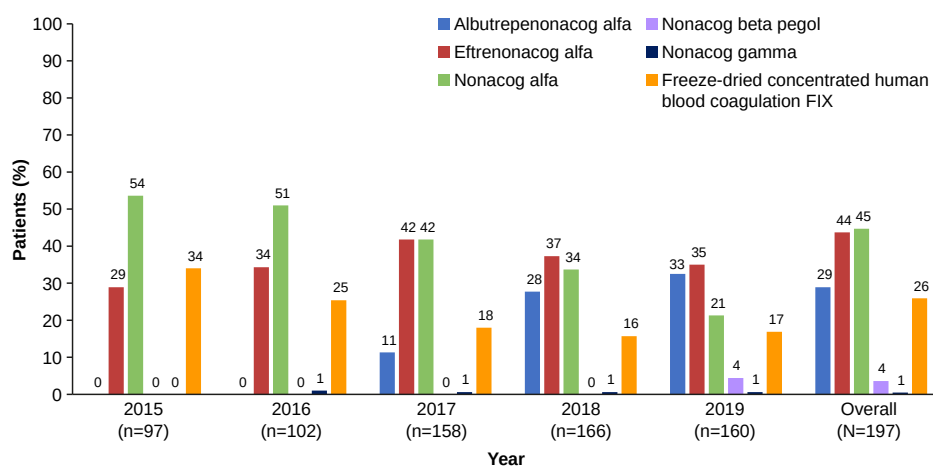
Table 1 Patient Age and Comorbidities

Characteristics	Total (N=197)
Age (continuous)	
Mean (SD)	30.6 (21.4)
Median (range), years	25.0 (<1.0–87.0)
Age category, years, n (%)	
0–11	36 (18.2)
12–19	38 (19.3)
20–64	106 (53.8)
65–74	10 (5.1)
≥75	7 (3.6)
Comorbidities, n (%)	
HIV	34 (17.3)
Viral hepatitis	82 (41.6)
Chronic viral hepatitis B	21 (10.7)
Chronic viral hepatitis C	76 (38.6)
Fibrosis and cirrhosis of liver	7 (3.6)
Malignant neoplasm of liver and intrahepatic bile ducts	20 (10.2)

Abbreviation: SD, standard deviation.

Prevalence of Joint Damage During the Study Period and Bleeding During Hospitalization

Overall, 58.9% of patients had documented joint damage (Table 3), which was highest (76.3%) for those aged 12 to 19 years (Supplementary Table S2). The most frequent type of joint damage was hemophilic arthritis, based on physicians' recorded clinical assessment (Table 3). A total of 30 (15.2%) patients experienced a bleeding event while hospitalized (ie, hospitalization with bleeding when bleeding was or was not the primary cause of hospitalization) (Table 4).

**Figure 2** Outpatient use of FIX concentrates.

Abbreviation: FIX, factor IX.

Table 2 Observed Treatment Duration and Annualized FIX Concentrate Dosage for Outpatients (All Ages)

Parameter	SHL FIX Concentrates			EHL FIX Concentrates		
	Nonacog Alfa (n=88)	Nonacog Gamma (n=1)	Freeze-Dried Concentrated Human Blood Coagulation FIX (n=51)	Albutrepenonacog Alfa (n=57)	Eftrenonacog Alfa (n=86)	Nonacog Beta Pegol (n=7)
Annualized FIX dosage, median (Q1, Q3), IU/kg	3157.7 (1362.4, 5206.8)	3181.4 (3181.4, 3181.4)	2092.9 (1172.9, 4077.0)	2156.7 (1484.9, 3103.1)	2447.4 (1905.0, 3401.0)	1507.4 (1386.5, 2148.0)
Observed treatment duration, n (%)						
<6 months	22 (21.2)	0 (0.0)	32 (41.6)	23 (32.4)	39 (32.8)	3 (42.9)
6 to <12 months	12 (11.5)	0 (0.0)	11 (14.3)	10 (14.1)	13 (10.9)	4 (57.1)
1 to <2 years	13 (12.5)	0 (0.0)	10 (13.0)	27 (38.0)	21 (17.7)	0 (0.0)
2 to <3 years	28 (26.9)	0 (0.0)	9 (11.7)	11 (15.5)	28 (23.5)	0 (0.0)
≥3 years	29 (27.9)	1 (100.0)	15 (19.5)	0 (0.0)	18 (15.1)	0 (0.0)

Abbreviations: EHL, extended half-life; FIX, factor IX; IU, international units; Q, quarter; SHL, standard half-life.

Table 3 Prevalence of Joint Damage in Diseases with a Frequency ≥1% Among the Study Population

Characteristics	Total Patients (N=197)
Patients with joint damage, n (%)	116 (58.9)
Prevalence rate of joint damage per 100 patient-years, rate (95% CI)	18.1 (15.1–21.1)
Types of joint damage, n (%)	
Hemophilic arthritis	49 (24.9)
Foot arthralgia	17 (8.6)
Knee osteoarthritis	17 (8.6)
Gonalgia	16 (8.1)
Coxalgia	15 (7.6)
Ankle osteoarthritis	14 (7.1)
Knee joint hematoma	12 (6.1)
Osteoarthritis	11 (5.6)
Arthralgia	10 (5.1)
Joint hematoma	9 (4.6)
Elbow arthralgia	8 (4.1)
Elbow osteoarthritis	7 (3.6)
Gonarthrosis	7 (3.6)

(Continued)

Table 3 (Continued).

Characteristics	Total Patients (N=197)
Shoulder joint pain	7 (3.6)
Coxarthrosis	6 (3.1)
Hemarthrosis	5 (2.5)
Knee hemarthrosis	5 (2.5)
Ankle arthropathy	4 (2.0)
Arthritis	4 (2.0)
Coxitis	4 (2.0)
Gonitis	4 (2.0)
Podarthritis	4 (2.0)
Shoulder osteoarthritis	4 (2.0)
Elbow hematoma	3 (1.5)
Hand osteoarthritis	3 (1.5)
Arthrosis	2 (1.0)
Omarthritis	2 (1.0)
Polyarthralgia	2 (1.0)

Abbreviation: CI, confidence interval.

Table 4 Bleeding During Hospitalization

Characteristics	Bleeding During Hospitalization (n=197)
Patients with bleeding during hospitalization, n (%)	30 (15.2)
Incidence of bleeding during hospitalization per 100 patient-years, rate (95% CI)	1.5 (0.8, 2.1)
Length of hospitalization, days, median (Q1, Q3)	10.0 (5.0, 21.0)
Type of bleeding occurring in ≥1% of patients, n (%)	
Intramuscular hematoma	9 (4.6)
Cerebral hemorrhage	3 (1.5)
Coxalgia	3 (1.5)
Arthralgia	2 (1.0)
Upper gastrointestinal hemorrhage	2 (1.0)
Joint hematoma	2 (1.0)

Abbreviations: CI, confidence interval; Q, quarter.

Expenditures for FIX Concentrate Treatment

Median annual total health care expenditures per patient with regular replacement therapy increased yearly during the study period, from ¥10,365,761 (\$95,099 USD, conversion of 109 yen/USD)²⁵ in 2015 to ¥28,714,530 (\$263,436 USD) in 2019. Median annual expenditures for prescribed FIX concentrates per patient also increased, from ¥9,686,448

(\$88,866 USD) to ¥28,484,282 (\$261,324 USD) (Table 5). Median annual total health care and median annual prescribed FIX concentrate expenditures for each age category trended similarly (Supplementary Table S3). In those aged 20 to 64 years, total health care expenditures increased from ¥9,057,510 (\$83,096 USD) in 2015 to ¥31,898,870 (\$292,650 USD) in 2019. In the same age group, prescribed FIX concentrate expenditures increased from ¥8,673,434 (\$79,573 USD) in 2015 to ¥30,718,086 (\$281,817 USD) in 2019. The expenditures for prescribed FIX concentrates accounted for the majority of the annual total health care expenditures. The data indicate a trend for lower annual total health care expenditures for patients without versus with joint damage. Health care expenditures were not evaluated in the subgroups of patient with or without joint damage because of the small number of patients.

Switch from SHL to EHL FIX Concentrate

The pattern of switching for patients who converted from an SHL to an EHL FIX concentrate is shown in Table 6. Overall, 25.9% (n=51/197) of patients switched from an SHL to an EHL concentrate during the study period, with the highest proportion of patients switching from nonacog alfa to either albutrepenonacog alfa (n=23/51 [45.1%]) or eftrenonacog alfa (n=14/51 [27.5%]). The median amount of prescribed FIX IU decreased slightly (2%) after the switch (Figure 3). However, median total health care (Figure 4) and FIX concentrate (Figure 5) expenditures increased shortly thereafter.

Discussion

The results of this real-world study show that dispensed factor doses of EHL FIX concentrates are increasing in Japan, with an accompanying increase in health care expenditures that is likely to be associated with switching from an SHL to an EHL concentrate. The number of hospitals in the MDV system also increased in since 2017 (291 sites in 2015 to 384 sites in 2019). The increase in median expenditures observed in the current analysis was similar to findings from other studies in smaller populations of patients with hemophilia B in the United States.^{15,19} A US study conducted by Tortella et al found a 238% increase in median health care expenditures after patients switched from an SHL to an EHL

Table 5 Medical Expenditures in Patients Receiving Regular Replacement FIX Therapy by Study Year

Expenditure Type	Overall (N=168)	2015 (n=86)	2016 (n=90)	2017 (n=146)	2018 (n=149)	2019 (n=130)
Annual total health care expenditures, median (Q1, Q3), yen	¥20,093,665 (¥7,954,423, ¥31,326,935)	¥10,365,761 (¥5,065,074, ¥17,043,696)	¥13,308,781 (¥6,149,975, ¥26,581,710)	¥18,956,681 (¥8,341,550, ¥29,275,142)	¥21,331,168 (¥8,217,347, ¥37,118,850)	¥28,714,530 (¥10,573,040, ¥41,391,933)
Annual expenditures for FIX concentrate treatment, median (Q1, Q3), yen	n=168 ¥18,705,754 (¥7,655,935, ¥30,690,493)	n=86 ¥9,686,448 (¥4,460,298, ¥16,829,800)	n=90 ¥12,804,595 (¥5,999,560, ¥24,781,240)	n=146 ¥16,825,898 (¥8,053,903, ¥27,878,895)	n=149 ¥19,463,520 (¥6,949,984, ¥35,905,198)	n=130 ¥28,484,282 (¥9,883,072–¥41,194,739)
Annual total health care expenditures for patients with joint damage, median (Q1, Q3), yen	n=104 ¥22,059,315 (¥9,547,612, ¥35,802,181)	n=61 ¥9,766,301 (¥4,676,570, ¥18,752,942)	n=61 ¥16,434,886 (¥6,528,554, ¥28,223,020)	n=91 ¥20,659,786 (¥9,733,540, ¥31,669,251)	n=96 ¥22,198,381 (¥9,563,011, ¥40,879,831)	n=85 ¥31,096,276 (¥12,932,652, ¥43,423,400)
Total health care expenditure for patients without joint damage, median (Q1, Q3), yen	n=64 ¥14,910,763 (¥5,985,481, ¥27,275,686)	n=25 ¥10,853,772 (¥6,788,352, ¥12,603,928)	n=29 ¥11,645,120 (¥5,992,417, ¥22,446,319)	n=55 ¥12,939,182 (¥5,024,129, ¥26,002,797)	n=53 ¥19,051,056 (¥6,384,160, ¥30,691,728)	n=45 ¥25,579,335 (¥9,879,532, ¥36,820,198)
Number of MDV-contracted sites		286	338	375	408	433

Abbreviations: FIX, factor IX; Q, quarter.

Table 6 Switching from an SHL to an EHL FIX Concentrate

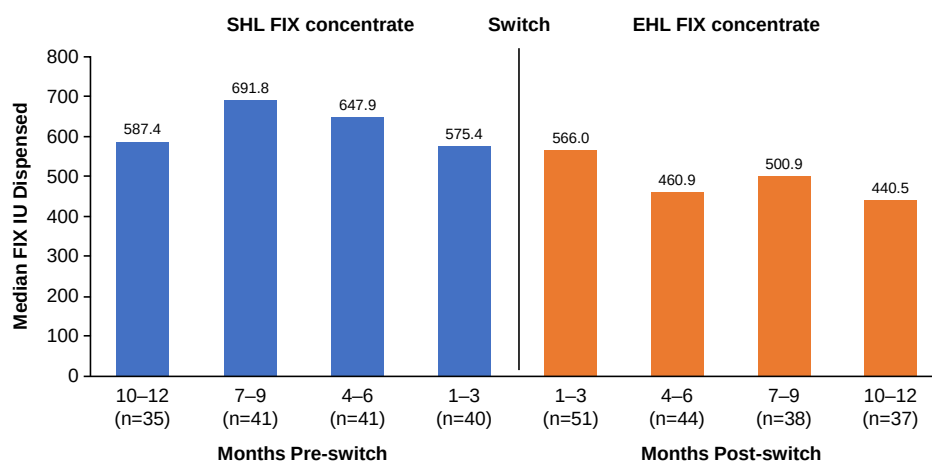
Switch Pattern, n (%)	Total (N=51)	Aged 0–5 y (n=3)	Aged 6–11 y (n=5)	Aged 12–19 y (n=16)	Aged 20–64 y (n=23)	Aged ≥65 y (n=4)
Freeze-dried concentrated human blood coagulation FIX to albutrepenonacog alfa	3 (5.9)	0 (0)	0 (0)	1 (6.3)	2 (8.7)	0 (0)
Freeze-dried concentrated human blood coagulation FIX to eftrenonacog alfa	10 (19.6)	0 (0)	0 (0)	2 (12.5)	7 (30.4)	1 (25.0)
Nonacog alfa to albutrepenonacog alfa	23 (45.1)	3 (100)	3 (60.0)	9 (56.3)	7 (30.4)	1 (25.0)
Nonacog alfa to eftrenonacog alfa	14 (27.5)	0 (0)	2 (40.0)	4 (25.0)	6 (26.1)	2 (50.0)
Nonacog alfa to nonacog beta pegol	1 (2.0)	0 (0)	0 (0)	0 (0)	1 (4.4)	0 (0)

Abbreviations: FIX, factor IX; EHL, extended half-life; SHL, standard half-life.

concentrate.¹⁹ Although the increase in drug expenditures is likely reflective of the increased uptake in EHL concentrates, it may be counterbalanced by improved treatment outcomes, such as quality of life,¹⁶ the assessment of which was beyond the scope of this study.

The current study highlighted a trend that indicates health care expenditures are higher for patients with joint damage compared with those with no joint damage. However, given the small number of patients hospitalized due to bleeding events, the hospital-related contribution to annual health care expenditures may be minimal. Further studies are necessary to fully elucidate this finding.

The retrospective design of the current analysis precludes more in-depth analysis of potential expenditure savings for various stakeholders, such as reduction in hospitalizations and the consequent decrease in associated hospital-related expenditures. In addition, this study was not designed to account for potential indirect expenditures incurred for bleeding events not requiring hospitalization that were treated in the community setting. Home therapy is allowed in Japan and is performed at the patient's discretion, even in the case of bleeding. Nevertheless, these data highlight the majority of medical expenditures for patients with hemophilia involve drug acquisition. In Japan, publicly funded financial support is available for patients with hemophilia, limiting out-of-pocket expenditures for patients. Although hemophilia B is generally associated with high expenditures in Japan,²⁶ as has been observed in other settings, such as the United States,²⁷ prophylaxis is considered the optimal care for treatment of hemophilia. As such, prophylaxis is the standard treatment approach to prevent bleeding events and preserve joint function.

**Figure 3** Median factor IUs dispensed before and after switching from an SHL to an EHL FIX concentrate.

Abbreviations: EHL, extended half-life; FIX, factor IX; IU, international units; SHL, standard half-life.

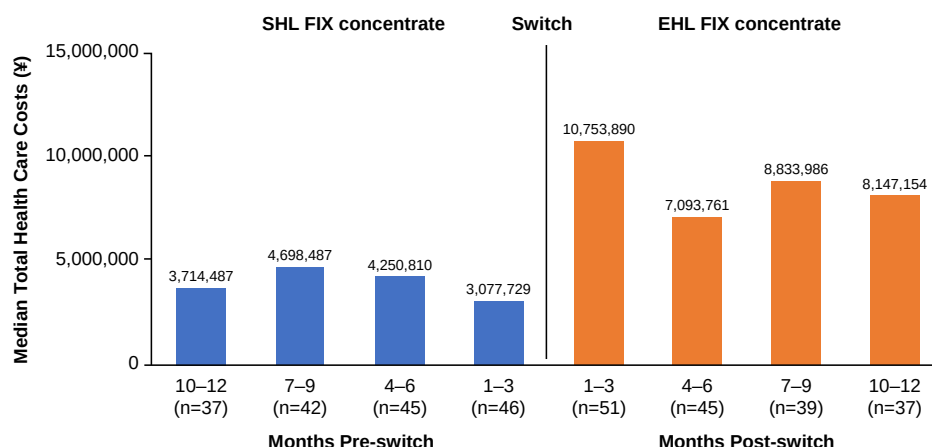


Figure 4 Median total health care expenditures before and after patients switched from an SHL to an EHL FIX concentrate.

Abbreviations: EHL, extended half-life; SHL, standard half-life.

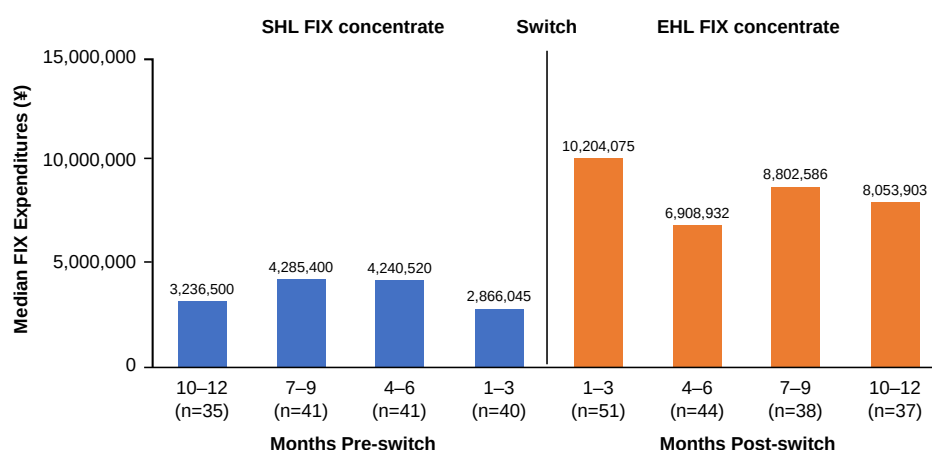


Figure 5 Median expenditures for prescribed FIX concentrates before and after patients switched from an SHL to an EHL concentrate.

Abbreviations: EHL, extended half-life; SHL, standard half-life.

In the current study, switching from an SHL to an EHL FIX concentrate was associated with a 2% decrease in the median number of FIX IU during the first 3 months after switching to an EHL concentrate, suggesting that more EHL FIX IU were dispensed than expected in the first 3 months after switching. Despite the minimal change in FIX IU dispensed in the first 3 months after switching, expenditures dramatically increased for health care (249% increase) and FIX concentrate (256% increase) during this period. In comparisons from 1 year before switching to 1 year after switching, a slight decrease in the median number of FIX IU occurred, although the median for health care and FIX concentrate expenditures still increased more than 2-fold. The drug price for eftrenonacog alfa, the first EHL concentrate in Japan, was 5% greater than the cost incurred if SHL concentrates were the standard routine prophylaxis regimen, and other EHL concentrates were priced accordingly. As a result, the drug price per IU of eftrenonacog alfa is approximately double the price of nonacog alfa.

When routine prophylaxis is implemented according to the package insert of each FIX concentrate and Japanese Society for Thrombosis and Hemostasis guidelines,¹⁴ the switch would be expected to reduce the dispensed FIX IU volume by approximately 50%, and the drug expenditure for EHL concentrates would be almost the same as that for SHL concentrates. However, the results of the current study show that the dispensed EHL FIX IU volume was approximately 70% to 80% that of the SHL FIX concentrate, with a corresponding approximate 2-fold increase in expenditures, in

the year after switching. Given that drug pricing takes into account the extended half-life of EHL FIX concentrates, higher treatment expenditures associated with switching from SHL FIX concentrates suggest higher and/or more frequent dosing than originally assumed, whereas SHL FIX concentrate prescribing trends remain relatively constant. Given that home therapy is allowed in Japan, it is possible that more EHL FIX concentrate prescriptions are dispensed than are actually administered, with the intention of stocking them at home after the switch, leading to an increase in medical costs. However, it is unlikely that this would have a significant impact on medical expenditures after switching.

The significant increase in expenditures reported in this study highlights the difference between actual prescribing trends in clinical practice and use of the standard treatment regimen as recommended in guidelines and package inserts. Although the clinical outcomes of switching have not been studied, the increased expenditure for treatment suggests that the intensity of treatment for each patient has increased or that previously undertreated patients are adequately treated after the switch. The standard regimen of regular prophylaxis treatment is stated in package inserts for EHL concentrates,^{5–7} not SHL concentrates. This difference may have improved prophylaxis treatment in patients with hemophilia B as treatment goals continue to advance. It is also possible that adherence to prophylaxis may have improved after switching.²⁸ These issues warrant further study. Understanding the impact on quality of life for patients who switch from an SHL to an EHL FIX concentrate may further enable quantification of the clinical benefits of switching.¹⁶

The diagnostic information in MDV pertained only to patients who required in-hospital tests or treatment. Consequently, the presence of comorbidities such as chronic hepatitis as well as the prevalence of joint damage may not reflect a real-world clinical setting. Chronic viral hepatitis C was present in a relatively large percentage (38.6%) of the study population; it is likely that many of these patients had sustained virologic response by antiviral therapy. However, the trends in patient demographics and baseline characteristics in this study followed those of the Nationwide Survey of Blood Coagulation Disorders 2020,²⁹ which reflects the overall picture of patients with hemophilia in Japan.

As stated, patients in the MDV database include only those receiving in-hospital treatment, which employs a per-diem payment system of diagnostic procedure combination. Characteristics such as the severity of comorbidities and/or joint damage may differ between patients managed within a hospital versus a clinic setting, thereby affecting treatment patterns and expenditures. In this study, weight was calculated based on the statistical average weight of Japanese individuals in the specified age groups, which could have led to an over- or under-estimate of annualized dosage of FIX concentrates. An additional limitation is potential underestimation of treatment-related and broader medical expenditures, as this analysis covers only expenditures for medications dispensed in the hospital setting, although patients do not typically receive prescriptions for FIX concentrates from multiple hospitals/clinics in the same time period. Each patient in the MDV database is assigned a unique identifier in a hospital; however, if a patient moves to another hospital also covered by the database, the patient would be recorded as two different patients. The evolution of treatment goals must be considered when interpreting differences in the estimated expenditures between SHL and EHL concentrates. However, information about the treatment goal is not included in the MDV database. Finally, patients with congenital hemophilia who had FIX inhibitors were excluded from this study; therefore, pre-switch and post-switch inhibitor rates were not evaluated. However, given the rarity of inhibitor in hemophilia B, a low incidence of FIX inhibitor would be expected.

Conclusions

This retrospective study found increased factor IU of EHL FIX concentrates dispensed for the treatment of Japanese patients with hemophilia B, along with an increase in associated medical expenditures for patients who switched from an SHL to an EHL FIX concentrate. The quality of prophylaxis treatment improved after EHL concentrates became available as treatment goals for hemophilia B continued to advance. Overall, these results provide insight into the treatment and expenditures of patients with hemophilia B in Japan and highlight the disease burden in patients requiring hospital care.

Data Sharing Statement

All the rights for the database used for the current study are reserved with Medical Data Vision. However, Pfizer Japan Inc. have a contract with Medical Data Vision to use this database and publish the results. The data may be made available upon request and are subject to a license agreement with Medical Data Vision.

Ethics Approval

This study was conducted in accordance with legal and regulatory requirements in Japan, and included anonymized claims data, with no personal patient data. Japanese Ethical Guidelines for Medical and Biological Research Involving Human Subjects do not apply to studies that use anonymized secondary data; consequently, this study was not reviewed by any institutional review board or ethics committee.

Acknowledgments

Medical writing and editorial assistance were provided by Courtney M. Cameron, PhD, of Engage Scientific Solutions and by Mia DeFino, MS, ELS, and Michael Morren, RPh, MBA, of Peloton Advantage, an OPEN Health company, and funded by Pfizer.

The affiliation for Leona E Markson was Pfizer Inc, Collegeville, PA, USA at the time of the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was sponsored by Pfizer.

Disclosure

Katsuyuki Fukutake has received honoraria from and/or served as a member of an advisory board or speakers' bureau for Bayer Yakuhin, Ltd., Chugai Pharmaceutical Co., Ltd., Fujimoto Pharmaceutical Co., Pfizer Japan Inc., Sanofi S.A., CellGenTech, Inc. and Takeda Co., Ltd. Kanae Togo, Linghua Xu, and Toshiyuki Karumori are employees of Pfizer Japan Inc. and may own stock/options in the company. José Maria Jimenez Alvir is an employee of Pfizer Inc. and may own stock/options in the company. Leona Markson was employed at Pfizer at the time of this study and previously retired from Merck and may hold stock/options or receive other retiree benefits from these companies. Ian Winburn is an employee of Pfizer Ltd. and may own stock/options in the company.

References

1. Franchini M. Current management of hemophilia B: recommendations, complications and emerging issues. *Expert Rev Hematol*. 2014;7(5):573–581. doi:10.1586/17474086.2014.947955
2. World Federation of Hemophilia. Report on the annual global survey 2021; 2022; Available from: <https://www1.wfh.org/publications/files/pdf-2324.pdf>. Accessed November 20, 2023.
3. Mancuso ME, Santagostino E. Outcome of clinical trials with new extended half-life FVIII/IX concentrates. *J Clin Med*. 2017;6(4):E39. doi:10.3390/jcm6040039
4. Fukutake K, Taki M, Matsushita T, et al. Postmarketing safety and effectiveness of recombinant factor IX (nonacog alfa) in Japanese patients with haemophilia B. *Haemophilia*. 2019;25(4):e247–e256. doi:10.1111/hae.13783
5. Pharmaceuticals and Medical Devices Agency. Refixia (nonacog beta pegol) Japanese regulatory review: report on the deliberation results; 2018; Available from: <https://www.pmda.go.jp/files/000238159.pdf>. Accessed February 8, 2023.
6. Pharmaceuticals and Medical Devices Agency. Idelvion (albutrepenonacog alfa) Japanese regulatory review: report on deliberation results; 2016; Available from: <https://www.pmda.go.jp/files/000221483.pdf>. Accessed February 8, 2023.
7. Biogen. Alprolix (eftrenonacog alfa); 2016; Available from: <https://www.pharmacodia.com/yaodu/html/v1/biologics/1e5e1435c95e420a1cd34d3202769c18.html>. Accessed February 8, 2023.
8. Pelland-Marcotte MC, Carcao MD. Hemophilia in a changing treatment landscape. *Hematol Oncol Clin North Am*. 2019;33(3):409–423. doi:10.1016/j.hoc.2019.01.007
9. Mannucci PM. Hemophilia therapy: the future has begun. *Haematologica*. 2020;105(3):545–553. doi:10.3324/haematol.2019.232132
10. Collins PW. Personalized prophylaxis. *Haemophilia*. 2012;18(suppl 4):131–135. doi:10.1111/j.1365-2516.2012.02838.x
11. Kavakli K, Smith L, Kuliczowski K, et al. Once-weekly prophylactic treatment versus on-demand treatment with nonacog alfa in patients with moderately severe to severe hemophilia B. *Haemophilia*. 2016;22(3):381–388. doi:10.1111/hae.12878
12. Powell JS, Pasi KJ, Ragni MV, et al. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. *N Engl J Med*. 2013;369(24):2313–2323. doi:10.1056/NEJMoa1305074

13. Mancuso ME, Lubetsky A, Pan-Petes B, et al. Long-term safety and efficacy of rIX-FP prophylaxis with extended dosing intervals up to 21 days in adults/adolescents with hemophilia B. *J Thromb Haemost*. 2020;18(5):1065–1074. doi:10.1111/jth.14778
14. Japanese Society for Thrombosis and Hemostasis. Hemostasis treatment guidelines for hemophilia patients without inhibitors; 2013; Available from: https://www.jsth.org/wordpress/wp-content/uploads/2015/04/03_inhibitor_H1_B.pdf. Accessed January 18, 2022.
15. Tortella BJ, Alvir J, McDonald M, et al. Real-world analysis of dispensed IUs of coagulation factor IX and resultant expenditures in hemophilia B patients receiving standard half-life versus extended half-life products and those switching from standard half-life to extended half-life products. *J Manag Care Spec Pharm*. 2018;24(7):643–653. doi:10.18553/jmcp.2018.17212
16. Cortesi PA, D'Angiolella LS, Lafranchi A, et al. Modern treatments of haemophilia: review of cost-effectiveness analyses and future directions. *Pharmacoeconomics*. 2018;36(3):263–284. doi:10.1007/s40273-017-0588-z
17. Guh S, Grosse SD, McAlister S, Kessler CM, Soucie JM. Healthcare expenditures for males with haemophilia and employer-sponsored insurance in the United States, 2008. *Haemophilia*. 2012;18(2):268–275. doi:10.1111/j.1365-2516.2011.02692.x
18. Machin N, Ragni MV, Smith KJ. Gene therapy in hemophilia A: a cost-effectiveness analysis. *Blood Adv*. 2018;2(14):1792–1798. doi:10.1182/bloodadvances.2018021345
19. Tortella BJ, Fogarty PF, Alvir J, et al. Real world data analysis of coagulation factor IX costs in specific patients with hemophilia B switching from standard half-life to extended half-life products [abstract]. *Blood*. 2016;128:4963.
20. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. *Lancet*. 2016;388(10040):187–197. doi:10.1016/s0140-6736(15)01123-x
21. Chhabra A, Spurdin D, Fogarty PF, et al. Real-world outcomes associated with standard half-life and extended half-life factor replacement products for treatment of haemophilia A and B. *Blood Coagul Fibrinolysis*. 2020;31(3):186–192. doi:10.1097/mbc.0000000000000885
22. The Japanese society for pediatric endocrinology. Available from: <http://jspe.umin.jp/medical/taikaku.html>. Accessed July 19, 2023.
23. General Counter of Official Statistics (e-Stat). National health and nutrition survey: average and standard deviation of height and weight. Available from: <https://www.e-stat.go.jp/dbview?sid=0003224177>. Accessed July 19, 2023.
24. Ministry of Health Labor and Welfare. Various information of medical fee. Available from: <https://shinryohoshu.mhlw.go.jp/shinryohoshu/>. Accessed July 20, 2023.
25. Bank of Japan. Transactions on the Tokyo foreign exchange market (in 2019); 2020; Available from: <https://www.boj.or.jp/statistics/market/forex/fxdaily/ex2019.pdf>. Accessed July 20, 2023.
26. Shirahata A. Treatment for hemophilia from the economic points of view. *Jpn J Thromb Hemost*. 2021;32(1):55–63. doi:10.2491/jjsth.32.55
27. Buckner TW, Bocharova I, Hagan K, et al. Health care resource utilization and cost burden of hemophilia B in the United States. *Blood Adv*. 2021;5(7):1954–1962. doi:10.1182/bloodadvances.2020003424
28. Nagao A, Bingo M, Yamaguchi T, Fukutake K. Real-world use of albutrepenonacog alfa, A recombinant coagulation factor IX albumin fusion protein, for personalized prophylaxis in Japanese individuals with hemophilia B: a case series. *Cureus*. 2023;15(1):e33573. doi:10.7759/cureus.33573
29. Japan Foundation for AIDS Prevention. National survey of blood coagulation disorders; 2021; Available from: <https://api-net.jfap.or.jp/library/project.html>. Accessed February 8, 2023.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Magnitude of Red Blood Cell Alloimmunization Among Pregnant Women Attending Antenatal Care at Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia

Tamirat Ersino Kebamo, Abinet Tantu Kombe, Temesgen Eticha, Mesay Arkew, Getachew Nigussie Bolado, Tadele Lankrew Ayalew, Kassahun Haile & Getachew Alemu Walano

To cite this article: Tamirat Ersino Kebamo, Abinet Tantu Kombe, Temesgen Eticha, Mesay Arkew, Getachew Nigussie Bolado, Tadele Lankrew Ayalew, Kassahun Haile & Getachew Alemu Walano (2023) Magnitude of Red Blood Cell Alloimmunization Among Pregnant Women Attending Antenatal Care at Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia, Journal of Blood Medicine, , 663-669, DOI: [10.2147/JBM.S440952](https://doi.org/10.2147/JBM.S440952)

To link to this article: <https://doi.org/10.2147/JBM.S440952>



© 2023 Kebamo et al.



Published online: 22 Dec 2023.



Submit your article to this journal



Article views: 201



View related articles



View Crossmark data

Magnitude of Red Blood Cell Alloimmunization Among Pregnant Women Attending Antenatal Care at Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia

Tamirat Ersino Kebamo¹, Abinet Tantu Kombe¹, Temesgen Eticha¹, Mesay Arkew², Getachew Nigusie Bolado³, Tadele Lankrew Ayalew³, Kassahun Haile⁴, Getachew Alemu Walano¹

¹School of Medical Laboratory Sciences, College of Health Sciences and Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia; ²School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; ³School of Nursing, College of Health Sciences and Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia; ⁴School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wolkite University, Wolkite, Ethiopia

Correspondence: Tamirat Ersino Kebamo, School of Medical Laboratory Sciences, College of Health Sciences and Medicine, Wolaita Sodo University, P.O. Box: 138, Wolaita Sodo, Ethiopia, Tel +251919675172, Email tamiratersino128@gmail.com

Background: Maternal red cell alloimmunization occurs when a woman's immune system becomes sensitive to unfamiliar red blood cell antigens. This leads to the production of alloantibodies, which can have serious implications for the fetus and newborn. However, there is a lack of comprehensive information about the extent of red cell alloimmunization in underdeveloped countries like Ethiopia. Therefore, this study aimed to determine the magnitude of red cell alloimmunization among pregnant women attending antenatal care at Wolaita Sodo University Comprehensive Specialized Hospital from September 01 to November 30, 2022.

Methods: In this institutional-based cross-sectional study, 422 pregnant women were participated and recruited using a systematic random sampling technique. Data on sociodemographic characteristics, obstetric history, and other clinical information were collected using structured questionnaires through face-to-face interview. Blood grouping and indirect antihuman globulin tests were performed. The relationship between red cell alloimmunization and the independent variables was determined using the chi-square test. P-value <0.05 was considered statistical significance.

Results: In this study, the blood group distributions among the participants were as follows: O, 177 (41.9%); A, 124 (29.4%); B, 76 (18%); and AB, 45 (3.86%). Among the pregnant women included in the study, a total of 51 (12.08%) were identified as RhD-negative. Out of these RhD-negative women, 5 (9.8%) were found to have developed alloimmunization with red blood cell antigens. Miscarriage and post-partum hemorrhage were found to be important factors associated with the occurrence of red cell alloimmunization in these women.

Conclusion: This study showed that one out of ten pregnant women was alloimmunized. Therefore, antenatal blood grouping and indirect antihuman globulin screening should be performed routinely to manage and minimize the undesirable outcomes of alloimmunization during pregnancy.

Keywords: alloimmunization, pregnant women, indirect antihuman globulin test

Introduction

Alloimmunization occurs when the immune system reacts to foreign antigens after being exposed to cells or tissues with different genetic characteristics. This immune response can happen during pregnancy or in individuals who receive frequent blood transfusions, such as those with hemoglobinopathies, myelodysplastic syndromes, chronic renal disease, and other medical conditions. Alloimmunization can be triggered by various factors, including human white blood cell antigens and red blood cell antigens.¹ Red blood cell alloimmunization among pregnant women range from 0.4% to 2.7% globally.²⁻⁴

The most clinically relevant blood group antigen present on the surface of red blood cells is still the ABO and Rhesus (Rh) blood group system.⁵ Rh alloimmunization happens when the immune system of the mother becomes sensitive to the RhD erythrocyte surface antigens, but it also refers to sensitization to other Rh antigens, including c, C, E and e.⁶ Alloimmunization, a process that produces a template for the production of antibodies as well as small amounts of antibodies against the RhD antigen, can occur in a mother who is D-negative when fetal D-positive cells are present in her circulation.^{2,7}

Although it can happen at any point in a pregnancy, the third trimester and labour are when it happens most frequently. It can occur after pregnancy-related occurrences that are linked to fetomaternal hemorrhage, such as terminations, late miscarriages, antepartum hemorrhage, abdominal trauma, ectopic pregnancy, intrauterine death, and stillbirth. These events include chorionic villus sampling, amniocentesis, and external cephalic version.⁷

When a woman's immune system becomes sensitive to unfamiliar red blood cell antigens, which results in the production of alloantibodies, these antibodies develop as a result of the immunological reaction to the initial exposure to the same antigen after a prior transplant, transfusion, or pregnancy. During birth or other trauma, newborn or fetal red cells in pregnant women enter the maternal circulation and lead to maternal red cell alloimmunization. If the fetus is positive for the relevant red blood cell surface antigens, the resultant antibodies frequently cross the placenta during subsequent pregnancies from sensitized women to the fetus, causing hemolysis of fetal red blood cells and anemia.^{8,9}

All pregnant women should be typed and screened to assess alloantibodies and for any unexpected RBC antibodies using indirect antiglobulin test is recommended for all pregnant women.¹⁰ The detection of Rh alloantibodies in pregnant women who are RhD-negative is crucial for predicting and treating hemolytic disease among newborns in resource limited nations.¹¹ However, there is currently inadequate prevention of alloimmunization following potentially sensitizing events and during medical termination of pregnancy in RhD-negative women. One of the reasons for this is the lack of sufficient data and information management regarding prior pregnancies. Additionally, crucial details regarding pregnancy termination are often missing from patients' medical records.⁷ Although various studies have been conducted throughout the world, there was a paucity of information on alloimmunization in our setting. Thus, this study aimed to assess the magnitude of red cell alloimmunization among pregnant women receiving antenatal care at Wolaita Sodo University Comprehensive Specialized Hospital.

Methods and Materials

Study Design, Period and Area

This institutional-based cross-sectional study was conducted from September 01 to November 30, 2022, at the Comprehensive Specialized Hospital of Wolaita Sodo University. The Hospital is located in Southern nations' nationalities and populations away from 328 km from Addis Ababa, Ethiopia. The altitude of the town is 1200–2950 m above the sea level.

Data Collection and Laboratory Methods

Study Participants who had communication problems, mental health issues, or were taking anti-D prophylaxis were excluded from the study. During the study period, all voluntarily pregnant women who attended prenatal care clinics were included. After obtaining written informed consent, data on sociodemographic characteristics, obstetric history, and other clinical information were collected through interviews with participants using structured questionnaires.

The test tube method was used to determine the study participants' ABO and Rh blood type using ABO and monoclonal IgM anti-D reagents. Blood samples collected from the study participants were washed four times with normal saline, and a 5% red cell suspension was prepared. Cell suspension mixed with known antiserum in a test tube; the mixture is incubated at room temperature and centrifuged. Finally, agglutination was observed both macroscopically and microscopically.¹² To detect alloimmunization, an indirect antihuman globulin test was performed.¹³ Serum from each participant was mixed with 5% red blood cell suspension prepared from known O-positive individuals. Agglutination was then seen after the mixture was centrifuged for 1 minute at 3000 revolutions per minute (RPM). The mixture was incubated at 37 °C for 30 minutes if it did not agglutinate. After incubation, the samples were again

centrifuged and checked for agglutination. If agglutination was not observed, the mixture was washed with normal saline four 4 times, and antihuman globulin reagent was added after completely discarding the last wash supernatant, followed by centrifugation for 1 min at 3000 RPM. Results were observed macroscopically and microscopically. Coombs control cells were added to all agglutination-negative samples to confirm the true negativity.

Data Management and Quality Assurance

All quality assurance programs were taken to ensure data quality. For accuracy and consistency, the questionnaire's English translation was made into the native tongue of Wolayitsa, then back into English. Before beginning the actual data collection, the questionnaire was pretested and the data collectors received training. The collection, handling, processing, and storage of samples were all done in accordance with standard operating procedures (SOP). Known ABO and Rh blood groups were utilized to test the ABO and Rh blood grouping reagents, and Coomb's control cells were employed to control the indirect antihuman globulin test quality.

Data Analysis and Interpretation

The data were checked for completeness and its consistency. Then, data were entered into Epidata version 3.1 (Epidata Association, Odense, Denmark) and then exported to the Statistical Package for Social Sciences (SPSS) version 25 software (IBM Corporation, USA) for analysis. To ascertain the frequency and percentage of the variables, categorical data were analyzed using the chi-square test to see if red cell alloimmunization was associated with the independent factors. P-value <0.05 was considered statistical significance.

Ethical Consideration

This study was carried out according to the Declaration of Helsinki. Ethical clearance was obtained from the Ethical Review Committee of Wolaita Sodo University, College of Health Science and Medicine (reference number: CSHM/ER/01/15). A support letter from the Chief Research and Community Service Director Office of the College was submitted to Wolaita Sodo University Comprehensive Specialized Hospital and written permission was obtained. Written informed consent was obtained from each study participant after explaining the objective and procedures of the study. For participants below 18 years old, assent was obtained from the participant, and consent was signed by their husband or parents. The study participants were identified by codes rather than individual name or identifiers to maintain data confidentiality, and unauthorized individuals were unable to access the collected data. Antihuman globulin test positive results were sent to physicians working in antenatal care clinics for proper management.

Results

Socio-Demographic Characteristics of Study Participants

Data were collected from 422 participants with a 100% response rate. In age distribution, 194 (46%) respondents were aged 16–25 years. Concerning participants' religions, 294 (69.7%) respondents were protestant religious followers. Regarding educational level, most of participants attended preparatory and above educational levels. Looking at the residence status of all participants, 230 (54.5%) were from urban areas, as shown in (Table 1).

Obstetric and Clinical Characteristics of Study Participants

Regarding obstetric history, 47 women (11.1%) had a history of post-partum hemorrhage. Regarding pregnancy complications, 44 (10.4%) of the study participants had history of malaria infection and 49 (11.6%) had a reported history of bleeding. In addition, 361 (85.5%) pregnant women had no history of blood transfusion. Concerning previous history of abortion/miscarriage among the pregnant women, 44 (10.4%) had a history of abortion/miscarriage. Most gestational age of participants, 217 (51.5%) were in their third trimester, while 51 (12.1%) were in their first trimester of pregnancy. A total of 223 (64.8%) study participants responded that they had a history of health center delivery (Table 2).

Table 1 Socio-Demographic Characteristics of the Study Participants in Wolaita Sodo University

Variables	Catagories	Frequency (%)
Age (in years)	16–25	194 (46)
	26–35	171 (40.5)
	36–45	57 (13.5)
Religion	Orthodox	76 (18)
	Protestant	294 (69.7)
	Muslim	33 (7.8)
	Catholic	19 (4.5)
Residence	Urban	230 (54.5)
	Rural	192 (45.5)
Educational status	Illiterate	34 (8.1)
	Read and write	98 (23.2)
	Primary and secondary	64 (15.2)
	Preparatory and above	226 (53.6)

Note: Comprehensive Specialized Hospital, Southwest Ethiopia, 2022 (n = 422).

Table 2 Obstetric and Clinical Characteristics of Study Participants in Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia, 2022 (n = 422)

Variables	Catagories	Frequency (%)
History post-partum Hemorrhage	Yes	47 (11.1)
	No	375 (88.9)
Pregnancy complication	Malaria	44 (10.4)
	Bleeding	49 (11.6)
	No complication	329 (78.0)
Previous history of blood transfusion	Yes	61 (14.5)
	No	361 (85.5)
History of abortion/miscarriage	Yes	44 (10.4)
	No	378 (89.6)
History of previous stillbirth	Yes	32 (7.6)
	No	390 (92.4)
Previous place of labour	Home	121 (35.2)
	Health center/hospital	223 (64.8)
Gestational age	1st trimester	51 (12.1)
	2nd trimester	154 (36.5)
	3rd trimester	217 (51.4)

Blood Group Distribution and Maternal Alloimmunization of the Study Participants

Regarding the blood group profile, among the total study participants, 177 (41.9%) were found to be blood group O, followed by 124 (29.4%), 76 (18%), and 45 (3.86%) with blood group A, B, and AB, respectively. From the total study participants, 51 (12.08%; 95 CI, 8.8–14.7) were RhD-negative, and 5 (9.8%; 95 CI, 0.2–2.1) were antihuman globulin (IAT) positive (Table 3).

Association Between Red Cell Alloimmunization and Other Variables

The variables associated with red cell alloimmunization among pregnant women that had been analyzed in this study included post-partum hemorrhage, blood transfusion, pregnancy complications, abortion/miscarriage, stillbirth, place of labor, and gestational age. Accordingly, the history of post-partum hemorrhage ($p = 0.001$) and miscarriage ($p = 0.001$) showed a statistically significant association with red cell alloimmunization. Other variables did not showed statistically a significantly associated with RBC alloimmunization (Table 4).

Table 3 Distribution of ABO, Rh Blood Groups and Alloimmunization Status Among the Pregnant Women at Wolaita Sodo University Comprehensive Specialized Hospital Southwest Ethiopia, 2022 (n = 422)

Blood Group	O	A	B	AB	IAT-Positive
Rh D positive	158	105	72	36	0
Rh D negative	19	19	4	9	5
Total	177 (41.9%)	124 (29.4)	76 (18%)	45 (3.86)	5 (9.8%)

Abbreviation: IAT, Indirect Anti-human globulin test.

Table 4 Association Between Red Cell Alloimmunization and Variables at Wolaita Sodo University Comprehensive Specialized Hospital, Southwest Ethiopia, 2022 (n = 422)

Variables	Categories	Alloimmunization		P value
		Yes	No	
Post-partum hemorrhage	Yes	3	44	0.001*
	No	1	374	
Previous blood transfusion	Yes	1	60	0.7
	No	4	357	
Pregnancy complication	Malaria	1	43	0.5
	Bleeding	0	49	
	No complication	4	325	
History of abortion/miscarriage	Yes	5	40	0.001*
	No	0	377	
History of previous stillbirth	Yes	1	31	0.27
	No	3	387	
Previous place of labour	Home	0	121	0.1
	Health center/hospital	4	219	
Gestational age	1st trimester	1	50	0.41
	2nd trimester	1	153	
	3rd trimester	4	213	

Note: *Chi square test was used as test of association.

Discussion

The incidence of red cell alloimmunization is decreased in high socioeconomic countries; nonetheless, it is still an issues in sub-Saharan and poor socioeconomic countries like, Ethiopia.⁷ A crucial first step in minimizing pregnancy-related difficulties from RhD alloimmunization is studying the prevalence of RhD-negative women. Based on the obtained prevalence, this could help with the creation of screening and preventive management programs. The percentage of pregnant participants in our study who had RhD-negative blood was 12.08% (confidence interval 8.8–14.7). This is significantly higher than studies done in southwest and southeast Nigeria, which, respectively, found 5.5 and 4.5% of women of reproductive age with Rh D-negative factors.^{14,15} This difference might be due to variations in the ethnic or racial groups of the population. The current study differs from that of a study carried out in Gambella, Southwest Ethiopia, which found a Rh D-negativity rate of 19.37%.¹⁶ This indicates that the Rh D-negative distribution among pregnant women varied across Ethiopia. There is evidence that, in addition to regional, ethnic, and racial variations, the frequency of Rh D-negative individuals fluctuates over time within a single population in various parts of the same country.¹⁷

The overall prevalence of red cell alloimmunization in this study was 9.8% (confidence interval 0.2–2.2) which is consistent with a study done in Southwestern Uganda, which was 9.8%¹⁸ and study conducted in Oman, the Arabian Peninsula in southwestern Asia, reported 10% alloimmunization in pregnant women.¹⁹ In this study, the prevalence of alloimmunization was lower than that reported for Uganda (12.1%).²⁰ This might be due to the relatively reduced sample

size and differences in phenotype of population. Red cell alloimmunization in our study was much higher than previous reports from developed countries such as the Netherlands,²¹ Sweden²² and Southwest Saudi Arabia²³ with 1.2%, 0.5%, and 1.8%, respectively. This gap might be brought about by racial variations resulting from the heterogeneity of various populations, and probably due to the routine antenatal use of anti-D prophylaxis.

Alloimmunization against RBC antigens may occur following blood transfusion, miscarriage, post-partum hemorrhage, stillbirth, place of labour, and pregnancy complications.²⁰ However, in this study, miscarriage ($p = 0.001$) and post-partum hemorrhage ($p = 0.001$) were significantly associated with red cell alloimmunization. Other variables did not show any statistically significant differences. In contrast to the present study, there was a significant association between parity and place of labor with red cell alloimmunization, as reported by Jimma.²⁴ This variation might be due to the differences in the relatively small sample sizes used in this study. No significant difference was observed in alloimmunization status across the three trimesters. This is possibly due to the unequal proportions of participants across the trimester groups and the relatively small sample size.

This study should be interpreted in the light of the following limitations: Firstly, it does not include the antibody identification panel of cells to resolve specificities of antibodies. Also, screening of autoantibody was not done due to resource constraints.

Conclusions and Recommendations

In our study population, the total prevalence of red cell alloimmunization was 9.8%, which is much higher than the prevalences recorded in European nations like the Netherlands, Sweden, and southern Arabia and slightly lower than those reported in African nations like Uganda. This variety is probably brought about by racial and genetic differences throughout the globe, sample sizes, and study methodologies. In this overall prevalence of D-negative women was 12.08%. Miscarriages/abortions and post-partum hemorrhage were significantly associated with red cell alloimmunization. Only pregnant patients who visited the hospital were included in our study, which does not accurately reflect the prevalence of anti-D among the many Ethiopian women who do not have access to obstetric care. It is important to look into additional population-specific antigens that may contribute to the significant amount of unidentified antibodies found in the current study.

Acknowledgment

We express our sincere gratitude to Wolaita Sodo University for ethical sanctions for this study. Moreover, we place on record our wholehearted thanks to the heads and staff of obstetrics and gynecology, neonatal care unit, and clinical laboratory staff, Wolaita Sodo University Comprehensive Specialized Hospital, and those who consented to participate in the study.

Author Contributions

All authors contributed to the work that was in the ideation, study design, execution, data collection, analysis, and interpretation processes as well as in the writing, and editing of the manuscript. All authors have agreed on the approval of the final manuscript to be published in the current journal and to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors affirm that they have no conflict of interest for this work.

References

1. Mota MA. Red cell and human leukocyte antigen alloimmunization in candidates for renal transplantation: a reality.. *Rev Bras Hematol Hemoter.* 2013;35(3):160–161. doi:10.5581/1516-8484.20130046
2. Ngoma AM, Mutombo PB, Ikeda K, et al. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: a systematic review and meta-analysis. *Transfus Apheresis Sci.* 2016;54(2):296–302. doi:10.1016/j.transci.2015.10.017

3. Pahuja S, Gupta SK, Jain M, Pujani M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. *Blood Transfus.* 2011;9(4):388. doi:10.2450/2011.0050-10
4. Gothwal M, Singh P, Bajpayee A, et al. Red cell alloimmunization in pregnancy: a study from a premier tertiary care centre of Western India. *Obstet Gynecol Sci.* 2023;66(2):84–93. doi:10.5468/ogs.22190
5. Cheesbrough MJUK. *District Laboratory Practice in Tropical Countries, Part 1.* 2 ed. Cambridge University Press; 2000.
6. Duplantie J, Gonzales OM, Bois A, et al. Cost-effectiveness of the management of rh-negative pregnant women. *Obstet Gynaecol.* 2013;35(8):730–740.
7. Osaro E, Charles AT. Rh isoimmunization in Sub-Saharan Africa indicates need for universal access to anti-RhD immunoglobulin and effective management of D-negative pregnancies. *Int J Women's Health.* 2010;429–437. doi:10.2147/IJWH.S15165
8. Altuntas N, Yenicesu İ, Himmetoglu Ö, et al. The risk assessment study for hemolytic disease of the fetus and newborn in a University Hospital in Turkey. *Transfus Apheresis Sci.* 2013;48(3):377–380. doi:10.1016/j.transci.2013.04.021
9. Bricca P, Guinchard E, Bliem C. Management of feto-maternal red cell allo-immunizations. *Transfus Clin Bio.* 2011;18(2):269–276. doi:10.1016/j.traci.2011.01.005
10. Urbaniak S, Greiss M. RhD haemolytic disease of the fetus and the newborn. *Blood Rev.* 2000;14(1):44–61. doi:10.1054/blre.1999.0123
11. Jennifer W, Meghan D. Red Blood Cell Alloimmunization in the pregnant patient. *Transfus Med Rev.* 2018;32(4):213–219. doi:10.1016/j.tmr.2018.07.002
12. Liu KL, Li N, Li BJ, Peng JY, Shu XW, Yu YY. Clinical significance of anti-D IgG screening and titer detection in 286 RhD negative pregnant women. *Chinese J Contemp Pediatr.* 2009;11(3):185–187.
13. Gupta A, Mirza S, Khurana S, et al. Enigmatic weak D antigen: an experience in a Tertiary Care Hospital of East Delhi. *J Clin Diagn Res.* 2016;10(6):EC12–EC15. doi:10.7860/JCDR/2016/16278.7972
14. Samson A, Hajara B, Health P. Prevalence of Rhesus D-negative blood type and the challenges of Rhesus D immunoprophylaxis among obstetric population in Ogbomoso, Southwestern Nigeria. *Ann Trop Med Public Health.* 2016;9(1):1–5.
15. Okeke T, Ocheni S, Nwagha U, et al. The prevalence of Rhesus negativity among pregnant women in Enugu, Southeast Nigeria. *Niger J Clin Pract.* 2012;15(4):400–402.
16. Golassa L, Tsegaye A, Erko B, et al. High rhesus (Rh (D)) negative frequency and ethnic-group based ABO blood group distribution in Ethiopia. *BMC Res Notes.* 2017;10(1):1–5. doi:10.1186/s13104-017-2644-3
17. Pourfathollah A, Oody A, Honarkaran N. Geographical distribution of ABO and Rh (D) blood groups among Iranian blood donors in the year 1361 (1982) as compared with that of the year 1380 (2001). *Sci J Iran Blood Transfus Organ.* 2004;1(1):11–17.
18. Mbalibulha Y, Natukunda B, Okwi AL, et al. Alloimmunization to Rh Antigen (D, C, E, C, E) among pregnant women attending antenatal care in South Western Uganda. *J Blood Med.* 2022;Volume 13:747–752. doi:10.2147/JBM.S385737
19. Al-Dughaisi T, Al Harrasi Y, Al-Duhli M, et al. Red cell alloimmunization to Rhesus antigen among pregnant women attending a tertiary care hospital in Oman. *Oman Med Journal.* 2016;31(1):77. doi:10.5001/omj.2016.15
20. Mbalibulha Y, Muwanguzi E, Mugenyi GR, et al. Occurrence of anti-D alloantibodies among pregnant women in Kasere District, Western Uganda. *J Blood Med.* 2015. 125–129. doi:10.2147/JBM.S80977
21. Koelewijn J, de Haas M, Vrijkotte T, et al. Gynaecology. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. *Int J Obstetr Gynaecol.* 2009;116(10):1307–1314. doi:10.1111/j.1471-0528.2009.02244.x
22. Filbey D, Hanson U, Wesström G. The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. *AGOS.* 1995;74(9):687–692.
23. Sarhan MA, Saleh KA, Bin-Dajem S. Distribution of ABO blood groups and rhesus factor in Southwest Saudi Arabia. *Saudi Med J.* 2009;30(1):116–119.
24. Nair SP, Segni H, Kassim M. Maternal alloantibodies and foetal complications in Jimma specialized hospital, South-Western Ethiopia. *World J Pharm Res.* 2016;5(2):1130–1140.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Predictors of Intensive Care Admission Among Adult Patients with Sickle Cell Disease in Eastern Province of Saudi Arabia

Mortadah Als Salman, Zaenb Als Salman, Hussain Abduljaleel Alkhalifa, Aman N Alfaraj, Ali Alkhalifah & Qasem Almulihi

To cite this article: Mortadah Als Salman, Zaenb Als Salman, Hussain Abduljaleel Alkhalifa, Aman N Alfaraj, Ali Alkhalifah & Qasem Almulihi (2023) Predictors of Intensive Care Admission Among Adult Patients with Sickle Cell Disease in Eastern Province of Saudi Arabia, Journal of Blood Medicine, , 671-680, DOI: [10.2147/JBM.S435861](https://doi.org/10.2147/JBM.S435861)

To link to this article: <https://doi.org/10.2147/JBM.S435861>



© 2023 Als Salman et al.



Published online: 26 Dec 2023.



Submit your article to this journal [↗](#)



Article views: 154



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 2 View citing articles [↗](#)

Predictors of Intensive Care Admission Among Adult Patients with Sickle Cell Disease in Eastern Province of Saudi Arabia

Mortadah Alsaman¹, Zaenb Alsaman², Hussain Abduljaleel Alkhalifa³, Aman N Alfaraj³, Ali Alkhalifah⁴, Qasem Almulihi⁵

¹Department of Medicine, College of Medicine, King Faisal University, Alahsa, Saudi Arabia; ²Department of Family and Community Medicine, College of Medicine, King Faisal University, Alahsa, Saudi Arabia; ³Department of Medicine, King Fahad Specialist Hospital, Dammam, Saudi Arabia;

⁴Emergency Department, Eastern Health Cluster, Dammam, Saudi Arabia; ⁵Emergency Department, King Fahad University Hospital, Al Khobar, Saudi Arabia

Correspondence: Zaenb Alsaman, Departments of Family and Community Medicine, College of Medicine, King Faisal University, Po Box 3311, AlAhsa, 36346, Saudi Arabia, Tel +966545611633, Email Alsaman.zainab@hotmail.com

Purpose: Sickle cell disease (SCD) comprises a complex group of hematologic disorders that are collectively the most common monogenic disorder and are associated with increased risk of intensive care unit admission (ICU). The purpose of this study is to investigate factors that predict admission of adult patients with SCD to the ICU.

Patients and Methods: This was a cross-sectional study that enrolled adult patients with SCD from Saudi Arabia.

Results: A total of 107 patients with SCD, with a median age 31.9 ± 12.1 years, were evaluated retrospectively. Regarding predictors of ICU admission, patients who indicated a history of blood transfusions were at 8.047-fold higher risk of ICU admission (OR=8.047; 95% CI=2.392–27.07; $p=0.001$). Patients who started hydroxyurea were at least 3.071 times more likely to be admitted than those who did not (OR=3.071; 95% CI=1.164–8.104; $p=0.023$). We also observed three or more hospitalizations per year to be associated with increased risk of ICU admission (OR=3.393; 95% CI=1.285–8.960; $p=0.014$), with those making 3 to 5 visits annually having at least 10.4 times higher risk (OR=10.38; 95% CI=10.098–98.19; $p=0.041$) and those with 6 to 10 ER admissions having 18 times higher risk (OR=18.00; 95% CI=2.149–150.8; $p=0.008$). Finally, patients with high WBC were predicted to have at least 3.34 times higher risk of ICU admission (OR=3.337; 95% CI=1.131–9.846; $p=0.029$).

Conclusion: SCD is a multi-systemic disease associated with increased morbidity and mortality. Recognition of high-risk features in patients helps to eliminate subjectivity in ICU referral decision. Frequent hospitalization and emergency visits, multiple blood transfusions, and elevated white blood cell count were significantly associated with a higher rate of ICU admission despite hydroxyurea usage.

Keywords: sickle cell disease, intensive care unit, hydroxyurea

Introduction

Sickle cell disease (SCD) is a group of inherited red blood cell disorders caused by a mutation in the β -globin gene that results in the polymerization of hemoglobin S (HbS).¹ There are different forms of SCD, however the most prevalent and severe form is homozygous HbSS (sickle cell anaemia).¹ In Saudi Arabia, SCD affects 0.26% of the population, with another 4.2% having the sickle cell trait. The Eastern province has the highest prevalence, with 1.2% diseased and about 17% having the trait.²

Patients with SCD experience a wide range of clinical symptoms and consequences, such as vaso-occlusive crises, acute chest syndrome, chronic hemolytic anemia, and multiple organ failure. The majority of complications are manageable without need for critical care, but some have life-threatening effects and may require admission to the intensive care unit (ICU).^{2,3} Currently, a variety of therapeutic options are available including disease-modifying therapies like

hydroxyurea and simple or exchange blood transfusions, which can improve quality of life, reduce the complication rate, and possibly prolong survival. However, the only curative option is stem cell transplantation, and its use is limited due to a lack of suitable donors.^{1,4} Despite these advances in management, the life expectancy of adult SCD patients is approximately twenty years shorter than the general population, with half of the mortality occurring in the ICU.^{5,6}

Several studies have been conducted to determine predictors of ICU outcomes, with the need for renal replacement therapy, advanced age, fewer hospitalizations, and shorter stays in the ICU having all been linked to increased mortality rates in SCD patients.⁷ In addition, it has been reported that low hemoglobin levels, respiratory distress, and renal failure at the time of ICU admission are significant predictors of a complicated outcome.⁶ Ultimately, it is believed that reducing the need for ICU admission among SCD patients will lower mortality and unfavorable outcomes. Therefore, the purpose of the current study was to investigate factors that predict admission of adult patients with SCD to the ICU.

Materials and Methods

This was an observational retrospective cohort study conducted in Saudi Arabia's eastern province, where SCD is more common than in other regions. All homozygous sickle cell patients between the age of 18 and 65, who attended hematology clinics or were admitted to King Fahad Hospital or Prince Saud Bin Jalawi Hospital between June and December 2022 were included in the study.

Participants initially underwent a survey through an interview conducted by a trained team member to collect demographic and clinical characteristics including age, education level, frequency of hospitalization, emergency visits, blood transfusion, previous ICU admission, frequency of ICU admission, cause of ICU admission, using and awareness about hydroxyurea. Hospital's electronic database was used to collect baseline patient's laboratory data from the most recent outpatient visit before ICU admission including complete blood count, hemoglobin electrophoresis, lactate dehydrogenase (LDH), and kidney function tests. Of note, using hydroxyurea was considered if patients have been on it for at least 2 months.

The study was approved by King Fahad Hospital's Ethical Committee (KFHH RCA 33-42-2021). Also, the purpose of the study, the use of data, benefits of doing this research, the right to participate, and the confidentiality and anonymity of the data were explained to the participants and informed consent was taken.

Analyses were performed using the Statistical Package for Software Sciences (SPSS) version 26 (IBM Corporation, Armonk, New York). Descriptive statistics were computed and are reported as frequencies and proportions (%) for categorical variables or means and standard deviations for continuous variables. Univariate regression analysis was performed to determine predictors of ICU admission with corresponding odds ratio (OR) and 95% confidence interval (CI). Statistical significance was identified at $p < 0.05$.

Results

A total of 107 patients with sickle cell disease were reviewed. As described in Table 1, more than half (52.3%) were aged 30 years or less, and the majority were female (60.7%). Patients with bachelor's degrees constituted 40.2% of participants. The proportion with previous blood transfusions was 86.9%, with 30 (28%) who reported having more than 10 blood transfusions throughout their life. Around two-thirds (71%) were aware of hydroxyurea, but less than half (46.7%) had started using it. As regards hospitalizations, 11.2% had more than 10 hospitalizations per year, and 26.2% had that many emergency visits per year. A history of ICU admission was reported by 75.7%, and nearly one-third (31.8%) of participants had 2 to 5 times ICU admission per year. Among those with a previous ICU admission, 21% had received hydroxyurea. Moreover, the most common reason being acute chest syndrome (ACS) (49.4%) (Figure 1). The mean values of leukocyte count, hemoglobin level, and platelet were 10.4, 8.74, and 326, respectively. Other participants' baseline laboratory parameters are shown in Table 2.

Regarding predictors of ICU admission, univariate analyses revealed previous blood transfusions, hydroxyurea initiation, frequency of hospitalizations per year, frequency of emergency visits per year, frequency of blood transfusions throughout life, and high WBC to be independent significant predictors of ICU admission. Specifically, compared to patients without a history of blood transfusion, those who indicated a history of blood transfusions were at 8.047-fold higher risk of ICU admission (OR=8.047; 95% CI=2.392–27.07; $p=0.001$). Patients who started hydroxyurea were predicted to have at least 3.071 times

Table I Demographic and Clinical Characteristics of the Patients (n=107)

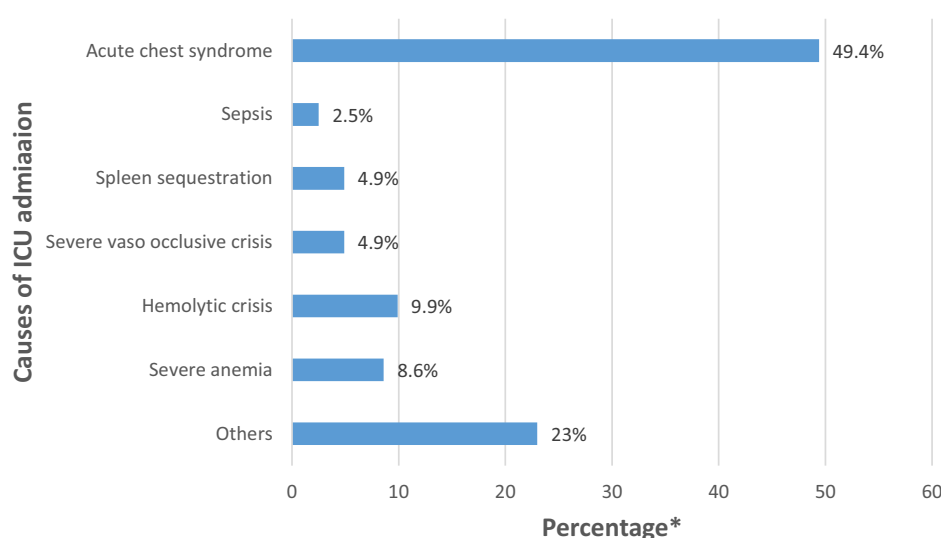
Variable	N (%)
Age in years (mean $\pm$ SD)	31.9 $\pm$ 12.1
• ≤ 30 years	56 (52.3%)
• > 30 years	51 (47.7%)
Gender	
• Male	42 (39.3)
• Female	65 (60.7)
Educational level	
• Illiterate	06 (05.6)
• Primary	11 (10.3)
• Intermediate	22 (20.6)
• Secondary education	43 (40.2)
• Bachelor's degree	22 (20.6)
• Master's degree	03 (02.8)
Complication and Comorbidity	
• Stroke	03 (02.8)
• Cholecystectomy	42 (39.3)
• Splenectomy	18 (16.8)
Previous blood transfusion	93 (86.9)
• Frequency of blood transfusions throughout life?	
• < 3 times	25 (23.4)
• 3–5 times	29 (27.1)
• 6–10 times	23 (21.5)
• > 10 times	30 (28.0)
Awareness about hydroxyurea	76 (71)
Hydroxyurea use	50 (46.7)
Frequency of hospitalization per year	
• < 3 times	55 (51.4)
• 3–5 times	27 (25.2)
• 6–10 times	13 (12.1)
• > 10 times	12 (11.2)

(Continued)

Table 1 (Continued).

Variable	N (%)
Frequency of emergency visit per year	
• <3 times	18 (16.8)
• 3–5 times	30 (28.0)
• 6–10 times	31 (29.0)
• >10 times	28 (26.2)
Previous ICU admission	81 (75.7)
• Frequency of ICU admission	
• One time	33 (30.8)
• 2–5 times	34 (31.8)
• More than 5 times	14 (13.1)
• No ICU Admission	26 (24.3)
Use of hydroxyurea during ICU admission (n=81)	17 (21.0)

greater risk than those who did not (OR=3.071; 95% CI=1.164–8.104; $p=0.023$). Meanwhile, three or more hospitalizations per year was associated with 3.393-fold higher risk (OR=3.393; 95% CI=1.285–8.960; $p=0.014$), 3–5 blood transfusions with 8.862-fold higher risk (OR=8.862; 95% CI=2.643–29.71; $p<0.001$), and >5 transfusions with 4.320-fold higher risk (OR=4.320; 95% CI=1.287–14.51; $p=0.018$). Likewise, compared to patients with less than 3 ER visits per year, those who had 3 to 5 visits had at least 10.4 times higher risk of ICU admission (OR=10.38; 95% CI=10.098–98.19; $p=0.041$), those with 6 to 10 ER admissions had 18 times higher risk (OR=18.00; 95% CI=2.149–150.8; $p=0.008$), and those with more than 10 ER admissions had 9.39 times higher risk (OR=9.391; 95% CI=1.092–80.78; $p=0.041$). Finally, patients with high WBC were predicted to have at least 3.34 times higher risk of ICU admission than those with low WBC (OR=3.337; 95% CI=1.131–9.846; $p=0.029$). (Table 3).


Figure 1 Causes of intensive care unit admission for sickle cell patients.

Note: *Percentage $\neq$ 100.

Table 2 Baseline Laboratory Data (n=107)

Variable	Mean $\pm$ SD
Leukocyte count, 10^9 /L	10.4 $\pm$ 5.04
Hemoglobin level, g/dL	8.74 $\pm$ 1.54
Platelet, 10^9 /L	326.0 $\pm$ 180.8
MCV, fL	78.3 $\pm$ 11.3
HbA, %	8.99 $\pm$ 16.5
HbA2, %	3.22 $\pm$ 1.08
HbS, %	74.4 $\pm$ 14.1
HbF, %	14.5 $\pm$ 6.89
LDH, U/L	415.1 $\pm$ 201.9
BUN, mmol/L	3.35 $\pm$ 2.35
Creatinine, μ mol/L	52.1 $\pm$ 34.1
Total Bilirubin, μ mol/L	45.2 $\pm$ 42.0
Direct Bilirubin, μ mol/L	11.4 $\pm$ 14.5
Albumin, g/L	37.4 $\pm$ 4.97
AST, IU/L	37.7 $\pm$ 19.6
ALT, IU/L	32.4 $\pm$ 26.0

Abbreviations: MCV, Mean corpuscular volume; LDH, lactate dehydrogenase enzyme; BUN, Blood Urea Nitrogen; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase.

Table 3 Univariate Logistic Regression Analysis

Variables	ICU Admission		Univariate	
	Yes N (%) ⁽ⁿ⁼⁸¹⁾	No N (%) ⁽ⁿ⁼²⁶⁾	OR (95% CI)	P-value
Age group				
• ≤30 years	40 (49.4%)	16 (61.5%)	Ref	0.283
• >30 years	41 (50.6%)	10 (38.5%)	640 (0.665–4.043)	
Gender				
• Male	33 (40.7%)	09 (34.6%)	Ref	0.578
• Female	48 (59.3%)	17 (65.4%)	0.770 (0.306–1.935)	
Previous blood transfusion				
• Yes	76 (93.8%)	17 (65.4%)	8.047 (2.392–27.07)	0.001 **
• No	05 (06.2%)	09 (34.6%)	Ref	
Splenicectomy				
• Yes	17 (21.0%)	01 (03.8%)	6.641 (0.839–52.58)	0.073
• No	64 (79.0%)	25 (96.2%)	Ref	

(Continued)

Table 3 (Continued).

Variables	ICU Admission		Univariate	
	Yes N (%) ⁽ⁿ⁼⁸¹⁾	No N (%) ⁽ⁿ⁼²⁶⁾	OR (95% CI)	P-value
Cholecystectomy				
• Yes	35 (43.2%)	07 (26.9%)	2.065 (0.782–5.457)	0.144
• No	46 (56.8%)	19 (73.1%)	Ref	
Hydroxyurea Use				
• Yes	43 (53.1%)	07 (26.9%)	3.071 (1.164–8.104)	0.023 **
• No	38 (46.9%)	19 (73.1%)	Ref	
• <3 times	36 (44.4%)	19 (73.1%)	Ref	
• ≥3 times	45 (55.6%)	07 (26.9%)	3.393 (1.285–8.960)	0.014 **
Number of emergency visit per year				
• <3 times	13 (16.0%)	05 (19.2%)	Ref	0.041 **
• 3–5 times	18 (22.2%)	12 (46.2%)	10.38 (1.098–98.19)	0.008 **
• 6–10 times	23 (28.4%)	08 (30.8%)	18.00 (2.149–150.8)	0.0401 **
• >10 times	27 (33.3%)	01 (03.8%)	9.391 (1.092–80.78)	
Frequency of blood transfusion				
• <3 times	13 (16.0%)	12 (46.2%)	Ref	
• 3–5 times	20 (24.7%)	09 (34.6%)	8.862 (2.643–29.71)	<0.001 **
• >5 times	48 (59.3%)	05 (19.2%)	4.320 (1.287–14.51)	0.018 **
WBC				
• Low	04 (04.9%)	02 (07.7%)	Ref	
• Normal	41 (50.6%)	19 (73.1%)	3.600 (0.518–25.00)	0.195
• High	36 (44.4%)	05 (19.2%)	3.337 (1.131–9.846)	0.029 **
Hemoglobin				
• Low	80 (98.8%)	26 (100%)	–	1.000
• Normal	01 (01.2%)	0	Ref	
Platelet				
• Low	13 (16.0%)	04 (15.4%)	Ref	
• Normal	39 (48.1%)	13 (50.0%)	0.991 (0.258–3.814)	0.990
• High	29 (35.8%)	09 (34.6%)	1.074 (0.405–2.851)	0.886
MCV				
• Low	40 (49.4%)	16 (61.5%)	Ref	
• Normal	37 (45.7%)	10 (38.5%)	1.480 (0.597–3.669)	0.397
• High	04 (04.9%)	0	–	–
LDH				
• Low	01 (01.3%)	0	Ref	
• Normal	24 (31.2%)	09 (37.5%)	–	–
• High	52 (67.5%)	15 (62.5%)	1.300 (0.499–3.387)	0.591

(Continued)

Table 3 (Continued).

Variables	ICU Admission		Univariate	
	Yes N (%) (n=81)	No N (%) (n=26)	OR (95% CI)	P-value
BUN				
• Low	09 (11.1%)	04 (15.4%)	Ref	
• Normal	65 (80.2%)	22 (84.6%)	1.313 (0.368–4.691)	0.675
• High	07 (08.6%)	0	–	–
Creatinine				
• Low	43 (53.1%)	13 (50.0%)	Ref	
• Normal	35 (43.2%)	12 (46.2%)	0.907 (0.087–9.478)	0.935
• High	03 (03.7%)	01 (03.8%)	1.029 (0.097–10.85)	0.981
Na				
• Low	04 (04.9%)	0	Ref	
• Normal	77 (95.1%)	26 (100%)	–	0.570
T. Bilirubin				
• Normal	14 (17.5%)	07 (26.7%)	Ref	
• High	66 (82.5%)	19 (73.1%)	1.737 (0.613–4.919)	0.299
D. Bilirubin				
• Normal	21 (26.3%)	11 (42.3%)	Ref	
• High	59 (73.8%)	15 (57.7%)	2.060 (0.818–5.189)	0.125
Albumin				
• Low	24 (31.2%)	04 (17.4%)	Ref	
• Normal	53 (68.8%)	19 (82.6%)	0.465 (0.143–1.515)	0.204
AST				
• Normal	27 (33.8%)	12 (46.2%)	Ref	
• High	53 (66.3%)	14 (53.8%)	1.683 (0.684–4.137)	0.257
ALT				
• Normal	50 (62.5%)	17 (65.4%)	Ref	
• High	30 (37.5%)	09 (34.6%)	1.133 (0.449–2.861)	0.791
	Mean ± SD	Mean ± SD		
HbA	9.22 ± 16.7	8.23 ± 16.4	0.996 (0.967–1.027)	0.806
HbA2	3.31 ± 1.12	2.94 ± 0.95	0.709 (0.442–1.136)	0.153
HbS	74.6 ± 14.1	73.9 ± 14.2	0.997 (0.966–1.030)	0.862
HbF	14.2 ± 7.13	15.5 ± 6.12	1.028 (0.962–1.099)	0.409

Notes: **Significant at p<0.05 level.

Discussion

Although considered the most common monogenic disorder, sickle cell disease comprises a complex group of hematologic disorders with multiple mechanisms involved in their pathophysiologies.^{8,9} The clinical heterogeneity and complexity of the disease are further modulated by fetal hemoglobin concentration and coincident alpha thalassemia. Thus, the

SCD disease course spans a spectrum from very benign to very severe, with corresponding increases in morbidity and mortality.^{10,11} A previous study revealed that half of the mortality of individuals with SCD occurs in the ICU, with acute chest syndrome being the most common reason for admission.⁶ In our study, we found that around two-thirds of participants had required ICU admission at least once in their lifetime, with acute chest syndrome the most common reason for admission. Interestingly, participants taking hydroxyurea were more likely to be admitted compared to those who did not. This raises a concern that while hydroxyurea is an effective treatment, SCD is a complex disease and a multimodal approach likely remains needed.^{8,12} However, it is important to note that our study did not address whether patients were on the maximum tolerated dose of hydroxyurea or not and at which age hydroxyurea was offered.^{13,14}

Several previous studies have reported concurring findings of increased risk of mortality among patients with SCD admitted to the ICU. In addition, five-year mortality is linked with a history of long ICU stay.^{15,16} Several predictors of increased mortality at ICU admission or during an ICU stay have been reported among patients with SCD; these include older age, renal replacement therapy, and frequent hospitalization use of inotropic support or mechanical ventilation.^{7,17} To our knowledge, the present work is the first study to address predictors of ICU admission among patients with SCD. Our result found that patients with three or more annual hospitalizations and emergency visits were at higher risk of ICU admission. Additionally, although red blood cell transfusion is an established treatment option for prevention of chronic organ damage, preoperative care, and acute complications of SCD, particularly acute chest syndrome, we found that history of previous blood transfusions of more than three was a significant predictor for ICU admission.^{16,18} These findings support a previous report of SCD patients having received exchange transfusions a few days prior to ICU admission; these patients may have an increased rate of alloimmunization and risk of delayed hemolytic transfusion reaction.^{19,20}

Sickle cell disease is characterized by heightened inflammation involving leukocytes, platelets, and endothelial cells, and also by inflammation resolution failure. We observed that patients with high white blood cell count were at higher risk to be admitted compared to those with low counts. Meanwhile, baseline thrombocytosis or thrombocytopenia were poorly correlated with ICU admission in our study, which is contrary to prior findings that thrombocytopenia is a poor prognostic indicator among ICU-admitted SCD patients.^{21,22}

This study illuminated characteristics that are predictors of ICU admission among patients with SCD. These include frequent hospitalization and emergency visits, multiple blood transfusions and elevated white blood cell count; moreover, admission increased despite hydroxyurea usage. The constellation of these factors could be translated into a scoring system that could be utilized to identify a high-risk group for early initiation of disease-modifying agents and to make early referrals to the ICU in order to achieve better outcomes. However, our study is limited by its retrospective nature and by physician-to-physician variability in making the decision for ICU admission. On this basis, prospective future studies are required, with the consideration of hydroxyurea usage duration and being on the maximum tolerated dose are crucial.

Conclusion

In conclusion, SCD is a multi-systemic disease associated with increased morbidity and mortality, and has a complex pathophysiology that mandates a multimodal treatment approach. Frequent hospitalization and emergency visits, multiple blood transfusions, and elevated white blood cell count were significantly associated with higher rate of ICU admission despite hydroxyurea usage. Recognition of these high-risk features in patients and establishment of severity scoring system can prompt initiation of a disease-modifying agent at a younger age and help to eliminate subjectivity in the timing of an ICU referral.

Data Sharing Statement

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical

The study was conducted in accordance with the Declaration of Helsinki and approved by King Fahad Hospital's Ethical Committee (KFHH RCA 33-42-2021). All participants provided informed consent before participation.

Acknowledgments

We would like to thank all participants for their collaboration.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not for fit sectors.

Disclosure

The authors report no conflicts of interest in this work.

References

- Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. In: *The Lancet*. Vol. 390. Lancet Publishing Group; 2017:311–323.
- Jastaniah W. Epidemiology of sickle cell disease in Saudi Arabia. *Annals of Saudi Medicine*. 2011;31(3):289–293. doi:10.4103/0256-4947.81540
- Houwing ME, de Pagter PJ, van Beers EJ, et al. Sickle cell disease: Clinical presentation and management of a global health challenge. In: *Blood Reviews*. Vol. 37. Churchill Livingstone; 2019.
- Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. *New England Journal of Medicine*. 2017;376(9):848–855. doi:10.1056/NEJMoa1609677
- Lubeck D, Agodoa I, Bhakta N, et al. estimated life expectancy and income of patients with sickle cell disease compared with those without sickle cell disease. *JAMA Netw Open*. 2019;2(11):e1915374. doi:10.1001/jamanetworkopen.2019.15374
- Cecchini J, Lionnet F, Djibré M, et al. Outcomes of adult patients with sickle cell disease admitted to the ICU: a case series. *Crit Care Med*. 2014;42(7):1629–1639. doi:10.1097/CCM.0000000000000316
- Al Khawaja SA, Ateya ZM, Al Hammam RA. Predictors of mortality in adults with Sickle cell disease admitted to intensive care unit in Bahrain. *J Crit Care*. 2017;42:238–242. doi:10.1016/j.jcrc.2017.07.032
- McGann PT, Ware RE. Hydroxyurea for sickle cell anemia: what have we learned and what questions still remain? *Curr Opin Hematol*. 2011;18(3):158–165. doi:10.1097/MOH.0b013e32834521dd
- Carden MA, Little J. Emerging disease-modifying therapies for sickle cell disease. In: *Haematologica*. Vol. 104. Ferrata Storti Foundation; 2019:1710–1719.
- Shah N, Beenhouwer D, Broder MS, et al. Development of a severity classification system for sickle cell disease. *ClinicoEconomics and Outcomes Research*. 2020;12:625–633. doi:10.2147/CEOR.S276121
- Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. *American Journal of Hematology*. 2012;87(8):795–803. doi:10.1002/ajh.23232
- Jagadeeswaran R, Rivers A. Evolving treatment paradigms in sickle cell disease. *Hematology*. 2017;2017(1):440–446. doi:10.1182/asheducation-2017.1.440
- Mak V, Davies SC. The pulmonary physician in critical care • Illustrative case 6: acute chest syndrome of sickle cell anaemia. *Thorax*. 2003;58(58):726–728. doi:10.1136/thorax.58.8.726
- John CC, Opoka RO, Latham TS, et al. Hydroxyurea dose escalation for sickle cell anemia in sub-saharan Africa. *New England Journal of Medicine*. 2020;382(26):2524–2533. doi:10.1056/NEJMoa2000146
- Hermans G, Van Aerde N, Meersseman P, et al. Five-year mortality and morbidity impact of prolonged versus brief ICU stay: a propensity score matched cohort study. *Thorax*. 2019;74(11):1037–1045. doi:10.1136/thoraxjnl-2018-213020
- Mekontso Dessap A, Fartoukh M, Machado RF. Ten tips for managing critically ill patients with sickle cell disease. *Intensive Care Med*. 2017;43(1):80–82. doi:10.1007/s00134-016-4472-7
- Tawfic QA, Kausalya R, Al-Sajee D, Burad J, Mohammed AK, Narayanan A. Adult sickle cell disease: a five-year experience of intensive care management in a university hospital in Oman. *Sultan Qaboos Univ Med J*. 2012;12(2):177–183. doi:10.12816/0003110
- Howard J, Hart N, Roberts-Harewood M, Cummins M, Awogbade M, Davis B. Guideline on the management of acute chest syndrome in sickle cell disease. *Br J Haematol*. 2015;169(4):492–505. doi:10.1111/bjh.13348
- Linder GE, Chou ST. Red cell transfusion and alloimmunization in sickle cell disease. In: *Haematologica*. Vol. 106. Ferrata Storti Foundation; 2021:1805–1815.
- Agbakou M, Mekontso-Dessap A, Pere M, et al. Nationwide retrospective study of critically ill adults with sickle cell disease in France. *Sci Rep*. 2021;11(1). doi:10.1038/s41598-021-02437-2
- Shome D, Jaradat A, Mahozi A, et al. The platelet count and its implications in sickle cell disease patients admitted for intensive care. *Indian J Crit Care Med*. 2018;22(8):585–590. doi:10.4103/ijccm.IJCCM_49_18
- Hogg PJ. TMX1: a new vascular thiol isomerase. *Blood*. 2019;133(3):188–190. doi:10.1182/blood-2018-11-887679

Journal of Blood Medicine

Dovepress

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>



Assessment of Hematological Profiles and Prognostic Role of Hemogram-Derived Novel Markers for Diabetes Mellitus and Its Complications Among Type 2 Diabetes Mellitus Adult Patients Attending Bishoftu General Hospital, Central, Ethiopia: A Comparative Cross-Sectional Study

Dereje Abebe Regassa, Girum Tesfaye Kiya, Regassa Alemu Kebede & Woyesa Beyene

To cite this article: Dereje Abebe Regassa, Girum Tesfaye Kiya, Regassa Alemu Kebede & Woyesa Beyene (2023) Assessment of Hematological Profiles and Prognostic Role of Hemogram-Derived Novel Markers for Diabetes Mellitus and Its Complications Among Type 2 Diabetes Mellitus Adult Patients Attending Bishoftu General Hospital, Central, Ethiopia: A Comparative Cross-Sectional Study, Journal of Blood Medicine, , 681-699, DOI: [10.2147/JBM.S435452](https://doi.org/10.2147/JBM.S435452)

To link to this article: <https://doi.org/10.2147/JBM.S435452>



© 2023 Regassa et al.



Published online: 27 Dec 2023.



Submit your article to this journal



Article views: 540



View related articles



View Crossmark data

Assessment of Hematological Profiles and Prognostic Role of Hemogram-Derived Novel Markers for Diabetes Mellitus and Its Complications Among Type 2 Diabetes Mellitus Adult Patients Attending Bishoftu General Hospital, Central, Ethiopia: A Comparative Cross-Sectional Study

Dereje Abebe Regassa¹, Girum Tesfaye Kiya², Regassa Alemu Kebede², Woyesa Beyene³

¹Department of Medical Laboratory Science, Wolkite University, Wolkite, Ethiopia; ²School of Medical Laboratory Sciences, Jimma University, Jimma, Ethiopia; ³Department of Medical Laboratory Science, Dire Dawa University, Dire Dawa, Ethiopia

Correspondence: Dereje Abebe Regassa, Department of Medical Laboratory Science, Wolkite University, P.O.Box 07, Wolkite, Ethiopia, Tel +251933864800; +251903036210, Email sifaanabebe@gmail.com; dereje.abeb@wku.edu.et

Background: Diabetes is a chronic metabolic syndrome that is a global public health problem. Studies have used hematological parameters and hemogram-derived markers as predictors of poor glycemic and microvascular complications status in diabetics. However, the tendency to use these parameters is not fully evaluated in our context, and the evidence is inadequate. This study aimed to assess the hematological profiles and prognostic role of hemogram-derived novel markers in diabetes mellitus and its complications among DM patients at Bishoftu General Hospital, Ethiopia.

Methods: A comparative cross-sectional study was conducted among 261 participants from June 15 to August 12, 2022. A systematic random sampling technique was used to select participants. Data were collected using structured questionnaires, physical measurements, checklists, and laboratory tests. Hematological parameters and fasting blood glucose levels were determined from blood using Sysmex-XN550 and Cobas C311 analyzers, respectively. Blood smear was used to check Hematology analyzer output, and to screen participants for malaria parasites. Collected data were entered into Epi-data 3.1 and exported to SPSS-25. Data were analyzed by Chi-square, Mann-Whitney *U*-test, Kruskal-Wallis test, Post hoc test, and ROC curve. A *P*-value <0.05 was considered statistically significant.

Results: Total WBC, neutrophils, Monocyte, NLR, MLR, MPVLR, and PLR were significantly higher in poor glycemic and complicated T2DM; meanwhile, measured RBC parameters, RBC indices values were significantly lower in poor glycemic and complicated T2DM. The NLR, MLR, MPVLR, PLR, and NLR, MLR, MPVLR, RPR values were identified as predictors of poor glycemic and complication status in diabetic patients, respectively.

Conclusion: Significant increment of some hematological parameters and hemogram-derived markers, and their role in predicting poor glycemic and microvascular complications were identified in diabetic patients. Routine screening of hematological parameters and use of hemogram-derived markers for monitoring of altered health status in DM is very important in the improvement of patient quality of life.

Keywords: hematological parameters, hemogram-derived markers, type 2 diabetes, Ethiopia

Introduction

Diabetes mellitus is a metabolic disorder that is chronic and progressive, caused by a variety of etiologies and characterized by persistent hyperglycemia.¹ Diabetes mellitus is usually classified into type 1 and type 2 diabetes mellitus; gestational diabetes and other set types of diabetes.^{1–3}

Type 2 diabetes is predominant, accounting for over 90% of cases worldwide.^{1,2} Globally, the prevalence of diabetes mellitus increased five-fold from 108 million in 1980 to 537 million in 2021.^{3,4} About 73 million people in the Middle East and North Africa and 24 million people in Sub-Saharan Africa have diabetes.⁴ According to IDF, the highest diabetes prevalence in 2021 was reported in Pakistan (30.8%), France (25.2%), Kuwait (24.9%), and Egypt 20.9%.⁴ Diabetes is also prevalent in Ethiopia, with a pooled prevalence of approximately 6.5%; about 3.3% of adults have diabetes.^{4,5}

About 541 million people of working age are at risk of developing type 2 diabetes. Diabetes is more common in urban than rural areas, and its prevalence is expected to be higher in middle-income countries compared with high- or low-income nations from 2021 to 2045.⁴

Global health-care costs due to diabetes increased from US\$232 billion in 2007 to US\$966 in 2021.⁴ Expenditures in sub-Saharan Africa are estimated to be US\$67.03 billion annually, and diabetes-related spending is estimated to be US\$7 trillion by 2025.⁶ About 7,108,145 deaths were recorded as shown by the (WHO) Mortality Database from diabetes complications collected in 108 countries from 2000 to 2016.⁷

Hyperglycemia mediates the excessive production of reactive oxygen and nitrogen species; the imbalance of production and removal mechanism of these reactive species causes vascular dysfunction, and damage to cellular proteins, membrane lipids, and nucleic acids.⁸ These can lead to macrovascular problems such as heart disease, stroke, and peripheral artery disease, and microvascular complications such as neuropathy, nephropathy, and retinopathy.⁹ Comorbidities shared in type 2 diabetic patients are hypertension, obesity, hyperlipidemia cardiovascular disease, chronic renal disease, and hematological disorders.¹⁰ Among hematological disorders, anemia frequently coincides with blood disorders with diabetes mellitus. There are multifaceted factors that prime diabetic patients to be anemic.^{9,11}

Chronic inflammation in diabetic mellitus patients causes WBC count to be elevated, which leads to diabetic macro- and microvascular complications.¹¹ The platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), mean platelet volume to lymphocyte ratio, and neutrophil-to-lymphocyte ratio (NLR) are also identified as potential markers of inflammation in various conditions, including tumors, cardiovascular conditions,^{12,13} and other diseases.¹⁴

Diabetic vascular complications also result in the alteration of platelet morphology and functions.^{9,11} In response to hyperglycemia, the neutrophil-derived S100 calcium-binding protein interacts with (RAGE) receptors, causing an increased level of IL-6, which results in thrombocytosis.¹⁵ Early prophecy of DM-related hyperglycemia and complications risk is very important for the well-being of diabetes patients and for preventing cardiovascular disease, slowing disease progression, and reducing mortality and morbidity.^{3,4}

Therefore, finding and using accessible, reliable, and inexpensive predictors from laboratory reports for early prevention and control of diabetes-related hyperglycemia and microvascular complications, especially in developing countries such as Ethiopia with constrained assets is very important. Hemogram indices such as mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Red cell distribution width (RDW), Mean platelet volume (MPV), platelet distribution width (PDW), platelet crit (PCT), P-LCR (platelet larger cell ratio) are useful tools for monitoring diabetes mellitus and could be used as possible indicators of its complications.¹¹

Besides, hemogram indices, hemogram-derived novel markers are conceived as tools in DM monitoring and as prospective forecasters of diabetes-related hyperglycemia and complications. Those hemogram-derived novel markers include Neutrophil-Lymphocyte ratio (NLR), Monocyte-Lymphocyte ratio (MLR), Red cell distribution width-platelet count ratio (RPR), Mean Platelet Volume to Lymphocyte Ratio (MPVLR), Platelet-Lymphocyte ratio (PLR), all of which can be used as predictive and prognostic markers in diabetes and its complications.^{12–14,16}

To date, only limited articles have studied the association between diabetic microvascular complications and the PLR, NLR, and MLR.^{17–22} Besides, a study done in Turkey reporting on MPVLR states that it can predict diabetic

complications.²³ Even though studies were conducted on the relationship between some hemogram-derived and DM, their findings are inconsistent. The associations of the RPR and MPVLR with DM-related poor glycemic control and complications are under investigation worldwide.

Though determination of hematological profile status and assessment of hemogram-derived markers have received boundless attention in the monitoring of diabetic patients because they have a fast prognostic role for diabetic poor glycemic control status and vascular complications in a simple, rapid, and inexpensive manner, to the best of our knowledge, no published article deals with the role of hemogram-derived markers with DM-related hyperglycemia and microvascular complications in our perspective.

Therefore, this study aimed to assess the hematological profile and determine the prognostic role of hemogram-derived markers for the prediction of DM-related hyperglycemia and microvascular complication status in type 2 diabetic patients in comparison with healthy controls attending Bishoftu General Hospital, central Ethiopia.

Methods and Materials

Study Design, Area, and Period

A hospital-based comparative cross-sectional study was conducted at Bishoftu General Hospital from June 15 to August 12, 2022. Bishoftu General Hospital is one of the hospitals in Oromia Regional State (ORS) and is located in Bishoftu town, 47 kilometers to the southeast of Addis Ababa. The town lies at an altitude of 1950 meters above sea level, at 9°N latitude and 40°E longitude.²⁴ There are two public hospitals, five health centers, two private hospitals, and ten private clinics, serving people in the catchment from preventive, basic curative to advanced medical services.

Bishoftu General Hospital was established in 1948 and currently serves approximately 1.2 million people with a variety of services.²⁵ The hospital offers chronic care, emergency services, ART services, surgical, dental, medical services, ophthalmology, pediatrics, gynecology and obstetrics, radiology, physiotherapy, Pathology services, pharmacy, laboratory, and other services. This study was conducted in the central laboratory of Bishoftu General Hospital which provides hematology and immunohematology, parasitology, microbiology, clinical chemistry, and serology services.

Study Population, Inclusion, and Exclusion Criteria

All adult patients already diagnosed with type 2 diabetes mellitus and who are on follow-up at the chronic care clinic of Bishoftu General Hospital were included in this study. Besides, all age and sex-matched healthy non-diabetic individuals who came to Bishoftu General Hospital during the study period for medical checkups, patient attendants were also included as the control group in this study.

To alleviate the impact of the confounding factors, based on information acquired from a medical record, interview of study participants, and through physicians' communication, the type 2 diabetic patients already diagnosed with chronic diseases such as renal failure, liver disease, hematological malignancy, patients with a history of infectious diseases such as HIV/AIDS, HBV, HCV, malarial infection, asthmatic, rheumatoid arthritis, patients who were severely ill, on anticoagulant therapy, on antiplatelet drugs, pregnant mothers, smokers, alcoholics, anemic patients, and those patients whose ages were below 15 and above 65 years old were not engaged in this study. In addition, the health condition of each control group was assessed, and individuals who had a history of malarial infection in the past two months, whose C-reactive Ab test was positive, smokers, and alcoholics were also excluded from this study.

Sample Size and Sampling Technique

The sample size was determined based on the two population mean formula using G-power software version 3.1. During sample size determination the 95% CI (2-tailed, $\alpha=0.05$), 80% power, a ratio of the control to case 1:1, a computed effect size (d) of 0.45, and 10% of non-respondents rate was considered. The mean and standard deviation ($X \pm SD$) of MPV for DM patients and control groups were taken from a study,²⁶ $[10.4 \pm 1.1]$ for type 2 diabetes, and $[9.9 \pm 1.1]$ for controls. To increase accuracy, the rule of thumb of Van Voorhis and Morgan²⁷ was used, and the determined sample size for T2DM was 174 (about 87 were poor glycemic control group, and 87 were good glycemic control group), and about 87 were healthy control group, and we had a total of 261 participants for this study.

Study participants were selected using a systematic random sampling method based on the order of follow-up visits attended. The sampling interval (the *k*th value) was obtained by dividing the estimated total number of the source population by the sample size (approximately 7). The hospital report per quarter estimated the number of people with type 2 diabetes at follow-up was 1260, and the calculated sample size for the cases was 174. Then, the *k*th value was $=1260/174=7$, and the first participant was selected by lottery method by writing the medical record numbers of the first seven participants on a separate piece of paper, and thereby the individual who came on the second number was selected as the first participant. Subsequently, patients with type 2 diabetes mellitus were interviewed face-to-face in between the service at every seven intervals of the order of visit.

Data Collection Procedure and Laboratory Methods

A total of 261 study participants from two groups were recruited for this study. The first group included 174 T2DM patients and the second group was 87 non-diabetic healthy controls. Based on the ADA-2017²⁸ criteria, the 174 T2DM study subjects were sub-grouped into 87 poor glycemic control type 2 diabetes mellitus group (whose FBG level >130 mg/dl with the conjunction of their worse clinical history), and the remaining 87 were good glycemic control type 2 diabetic patients (whose FBG level was 80 mg/dl to 130 mg/dl and their clinical histories were also good).

Checklists were used to extract clinical characteristics such as duration of DM illness, presence or absence of microvascular complications, type of oral hypoglycemic drugs, and fasting blood glucose levels in the last 2 months from patients' history on medical records ([Supplementary Material 1](#)). Diabetic patients fasting blood glucose readings for at least 3 months including the current reading were used for computing the average blood glucose level.

After the participants took a rest for more than 5 minutes, blood pressure (BP) measurement was taken from the upper arm of the left hand at the heart level by sphygmomanometer and stethoscope analogs twice simultaneously within 2-minute intervals, and reported by millimeter mercury (mmHg). For the participants whose BP difference was greater than 5 mmHg the BP measurement procedures were repeated. The stadiometer and weighing scale were used for the measurement of height (to the nearest 0.1 centimeters without shoes), weight (to the nearest 0.1 kg) without shoes, and wearing very light clothing; then BMI was computed by dividing the weight in kilograms by height in meters squared.

Waist circumference (WC) was measured with a non-stretchable tape at the midway between the least palpable inferior margin of the rib and the iliac crest in the participant's normal exhaled state. The hip circumference (HC) was also measured using the same tape around the widest portion of the buttocks, and then the waist-to-hip ratio (WHR) was computed by dividing WC in centimeters by HC. All anthropometric measurements were performed by trained clinical nurses according to the WHO anthropometric protocol. Anthropometric and blood pressure measurements were performed twice and the mean values were used for analysis.

After an interview (see [Supplementary Material 2](#) and [Supplementary Material 3](#)), record review, and anthropometric and blood pressure measurements taken by a trained clinical nurse, study subjects were sent to the central laboratory of Bishoftu Hospital, and then the vein of the antecubital fossa of the forearm was disinfected with 70% alcohol, and a tourniquet was applied as needed, then the required amount of blood was collected. A total of 5 mL venous blood sample was collected by vacutainer methods from each T2DM patient by a trained laboratory technologist in two different tubes; 3 mL of blood sample was taken into a K2-EDTA test tube for hematological parameters determination, blood smear preparation, and 2 mL into sodium fluoride test tube for glucose analysis.

The blood sample collected in a serum separator tube stayed for 15 minutes at room temperature; then serum was separated by centrifugation at 3600 rpm for 3 minutes using a Sorvall-ST-16 centrifuge, and the glucose level was determined. With the same procedure and precaution, 3 mL of venous blood was collected into a K2-EDTA test tube from each control participant for determination of hematological parameters, blood smear preparation, and C-reactive protein Ab test. A C-reactive protein test is a blood test used to measure the amount of CRP in the blood. This test was used to screen the inflammatory status of control groups and we use this test result as inclusion or exclusion criteria. We used the qualitative C-reactive protein test which is based on a latex agglutination principle that gives a positive or negative result.

Fasting blood glucose was determined from the serum sample prepared from an organ test tube by Cobas C311 (Roche system, Germany) following the photometric analysis principle, detecting glucose level in serum

spectrophotometrically at 340 nm.²⁹ A five differential automated hematological analyzer Sysmex XN550 (Sysmex, Germany) which works by the combination of hydrodynamically focused impedance measurement for RBC and platelet count, flow cytometer method for WBC differential count and photometric principle for hemoglobin determination was used.³⁰

Thin blood film was prepared, labeled, air-dried, and then stained by Wright stain to recheck the hematological parameters output of the hematological analyzer and to evaluate the morphology of blood cells, and it was also used to screen participants for malarial agents. Fasting blood glucose level, smear preparation and diagnosis, and hematological parameters determination were done at the central laboratory of Bishoftu General Hospital, and the result of each study participant was printed and attached to the request paper and recorded using laboratory result registration format.

Data Quality Assurance and Management

To ensure the quality of data, questionnaires and informed consent forms written in English were translated into the local language Afaan Oromo and Amharic, and retranslated into English for accuracy and consistency. Before the actual data collection, questionnaires were pretested on 5% of the total sample size (13 subjects who met the eligibility criteria) at Mojo General Hospital, which is located in East Shewa, Mojo town. Half-day training was given for four data collectors (two clinical nurses and two laboratory technologists), on study objectives, data collection procedures, and confidentiality of collected information to reduce technical and observation bias.

To maintain the quality of the socio-demographic, anthropometric, and clinical data, daily completeness and consistency were checked by on-site supervision of the data collectors during the study period. A code was used to maintain the confidentiality of study participants' test results, and records were kept in a secret, inaccessible place. Feedback and corrections were provided as needed based on the daily data collection process.

The manufacturers' instructions and standard operating procedures were followed during specimen collection, CBC analysis, fasting blood glucose determination, and blood film preparation and examination to maintain the quality of laboratory data. Thus, to avoid post-collection hemolysis, the blood sample was dispensed to the walls of a K2-EDTA test tube, gently inverted 8–10 times to mix properly, and hematological parameters were determined. The RBC morphology was critically observed under a microscope by using Wright-stained thin blood film and morphological classification of anemia was done.

Acceptance criteria of samples such as non-hemolysis, non-clotting, adequate sample volume, and sample collection criteria were critically followed. To avoid mix-up after collection, labeling was done on the sample holder and the request paper with the same identification code. Based on the Hospital laboratory protocol, low, normal, and high control materials were used for the hematology analyzer. Normal and pathological control materials were performed for the Cobas C311 analyzer when measuring glucose. Background readings were obtained daily to reduce background carry-over effects. Reagent expiration dates were checked before the analysis of patient samples. All laboratory assays were analyzed within 2 hours of sample collection, all laboratory test results were recorded and reported and samples were properly managed.

Statistical Data Analysis and Interpretations

Collected data were checked for completeness and consistency, entered into Epi-Data version 3.1 (Epi-Data Association, Denmark), and analyzed using Statistical Package for Social Sciences (SPSS) software version 25 (IBM SPSS Statistics, USA). Histograms and the Kolmogorov–Smirnov test were used to check the normality of the data distribution. Results for categorical variables were reported in frequency and percentage. For these categorical variables, statistical differences were checked by the chi-square test.

Continuous parameters were not normally distributed in the goodness-of-fit model test. Therefore, descriptive analyses were reported as median (interquartile range (IQR)). Mann–Whitney *U*-test and Kruskal–Wallis analysis were done to compare hematological parameters and hemogram-derived marker values between groups. The Kruskal–Wallis test comparison-wise and post-hoc were used to compare the values of hematological parameters and hemogram-derived markers between the three groups. The receiver operating characteristic curve (ROC) was constructed to determine the sensitivity, specificity, cutoff value, Area under the curve, PPV, and NPV of hemogram-derived new markers for

distinguishing the poor glycemic control from good glycemic control status and the discrimination ability of these parameters for vascular complication from non-complication status in diabetic patients. A P-value <0.05 was considered statistically significant.

Results

Socio-Demographic Characteristics of the Study Participants

Sociodemographic data showed that type 2 diabetic patients with poor or good glycemic control and healthy control study groups did not differ significantly in terms of age, sex, residence, marital status, and monthly income ($p>0.05$). The median (IQR) of age in T2DM with poor glycemic control was 33,^{28–40} good glycemic control was 32 (27–37%) and in healthy controls was 31^{27–38} ($p=0.82$). Most of the study participants were males, about 70 (80.5%) were from type 2 diabetes mellitus with uncontrolled glycemic control group, 69 (99.3%) were type 2 diabetes mellitus with glycemic controlled T2DM, and 66 (75.9%) were from healthy control group. However, those groups were different regarding their educational status and occupational status ($p<0.05$) (Table 1).

The Anthropometric and Clinical Characteristics of Study Participants

Regarding the anthropometric and clinical characteristics of the study participants, there were statistically significant differences between groups concerning BMI, WHR, SBP, and DBP ($p<0.001$) and for WC ($p=0.003$). The FBG level in

Table 1 Socio-Demographic Characteristics of Study Participants at Bishoftu General Hospital, Central Ethiopia, from June 15 to August 12, 2022 (n=261)

Variables	Categories	Poor Glycemic Level T2DM n(87)	Good Glycemic Level T2DM n(87)	Healthy Controls	p-value
Age	Media(IQR)	33(28–40)	32(27–37)	31(27–38)	0.82
Sex	M n(%)	70(80.5%)	69(79.3%)	66(75.9%)	0.74
	F n(%)	17(19.5%)	18(20.7%)	21(24.1%)	
Residence	Urban n(%)	74(85.1%)	73(83.9%)	68(78.2%)	0.44
	Rural n(%)	13(14.9%)	14(16.1%)	19(21.8%)	
Educational level	Unable to write and read n(%)	5(5.7%)	3(3.4%)	2(2.3%)	0.00
	Can write and read n(%)	4(4.6%)	7(8%)	2(2.3%)	
	Primary school n(%)	13(14.9%)	12(13.8%)	9(10.3%)	
	Secondary school n(%)	43(49.4%)	23(26.4%)	50(57.5%)	
	Higher education n(%)	22(25.3%)	42(48.3%)	24(27.6%)	
Occupational status	Unemployed n(%)	31(35.6%)	6(6.9%)	37(42.5%)	0.00
	Merchant n(%)	21(24.1%)	26(29.9%)	20(23%)	
	Farmer n(%)	12(13.8%)	13(14.9%)	7(8%)	
	Gov't employee n(%)	23(26.8%)	42(48.3%)	23(26.4%)	
Marital status	Single n(%)	31(35.6%)	28(32.2%)	33(37.9%)	0.98
	Married n(%)	36(41.4%)	39(44.8%)	35(40.2%)	
	Divorced n(%)	15(17.2%)	14(16.1%)	14(16.1%)	
	Widowed n(%)	5(5.7%)	6(6.9%)	5(5.7%)	

Notes: Data are presented by frequency and percentage.

Abbreviations: IQR - Interquartile range, T2DM - type 2 diabetes mellitus, ETB - Ethiopian birr, M - male, F - female, n - numbers or frequency.

Table 2 Anthropometric and Clinical Characteristics Distribution of Study Participants at Bishoftu General Hospital, Central Ethiopia, from June 15 to August 12, 2022 (n=261)

Variables	Categories	Poor Glycemic Control T2DM n(87)	Good Glycemic Control T2DM n(87)	Healthy Control n(87)	P-value
BMI (Kg/m ²)	Media(IQR)	24.7(22.6–26.0)	21.7(19.5–24)	20.3(19.1–22.03)	<0.001
WC (cm)	Media(IQR)	95(88–98)	89(86–94)	89(86–92)	0.003
WHR	Media(IQR)	0.96(0.90–0.99)	0.89(0.84–0.94)	0.87(0.83–0.92)	<0.001
SBP (mmHg)	Median(IQR)	150(144–156)	134(128–139)	131(126–135)	<0.001
DBP (mmHg)	Media(IQR)	109(104–114)	94(89–98)	90(87.0–92.0)	<0.001
FBG level (mg/dl)	Media(IQR)	150(144–155)	114.7(108–126.3)	–	<0.001
Duration (years)	Media(IQR)	10(6.0–11.0)	4(3.0–5.0)	–	<0.001
Presence of complication at least one type	Yes n(%)	45(51.7%)	–	–	–
	No n(%)	42(48.3%)	–	–	
Current Oral hypoglycemic therapy used by DM patients	Glibenclamide n(%)	17(19.5%)	24(27.6%)	–	–
	Metformin n(%)	46(52.9%)	43(49.4%)	–	
	Metformin +Glibenclamide n(%)	24(27.6%)	20(23%)	–	

Note: Data are expressed by frequency and percentage, median(IQR).

Abbreviations: BMI - body mass index, WC - waist circumference, WHR - waist to hip ratio, SBP - systolic blood pressure, DBP - diastolic blood pressure, FBS - fasting blood sugar, DM - diabetes mellitus.

poor glycemic control was higher than in good glycemic-controlled T2DM patients ($p < 0.001$). Among type 2 diabetic patients with uncontrolled glycemic levels, about 45 (51.7%) experienced at least one type of microvascular complication, and about 46 (52.9%) of diabetes patients with poor glucoregulation and 43 (49.4%) of diabetes patients with good glucoregulation had received oral metformin therapy (Table 2).

Distribution of Anemia Based on the Severity Among Type 2 Diabetic Patients Attending Bishoftu Hospital, Oromia, Central Ethiopia, from June 15 to August 12, 2022 (n=66)

The severity of anemia was assessed among poor and good glycemic control diabetic patients. Accordingly, among poor glycemic control about 60 patients were anemic of these 56.7%, 25.0% and 18.3% experienced moderate, mild, and severe anemia, respectively. Among good glycemic control, only 6 patients had anemia, of these about 50% and 50% experienced moderate and mild anemia, respectively (Figure 1).

Distribution of Morphological Type of Anemia Among Type 2 Diabetic Patients Attending Bishoftu Hospital, Oromia, Central Ethiopia, from June 15 to August 12, 2022 (n=66)

The morphological classification of anemia was also assessed among poor and good glycemic control type 2 diabetic patients. In poor glycemic control about 60 patients were anemic with 31.7% normocytic normochromic and 68.3% microcytic hypochromic type of anemia, respectively. In good glycemic control type 2 diabetic patients about 83.3% and 16.7% of patients experienced normocytic normochromic and microcytic hypochromic morphological types of anemia, respectively (Figure 2).

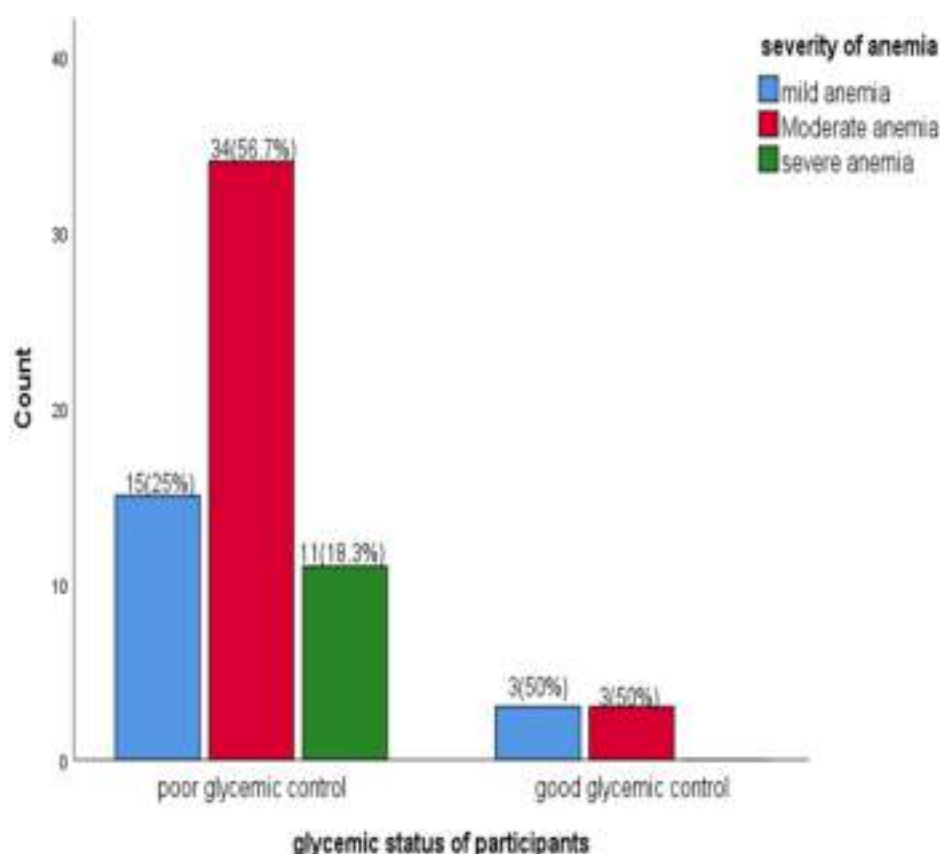


Figure 1 Distribution of anemia based on the severity among type 2 diabetic patients attending Bishoftu Hospital, Oromia, central Ethiopia, from June 15 to August 12, 2022.

Determination of Hematological Parameters and Hemogram-Derived Novel Markers Among Participants

There was no significant difference between poor glucoregulation, good glucoregulation, and healthy control groups in terms of platelet count, red blood cell width standard deviation, absolute eosinophils count, absolute basophils count, Immunoglobulin levels, and red blood cell width to platelet count ratio according to the Kruskal–Wallis test ($p > 0.05$).

The Kruskal–Wallis and post hoc comparison pairwise test also showed that the red blood cell width coefficient variation, total white blood cells, absolute neutrophils count, mean platelet volume, absolute monocyte, neutrophils to lymphocyte ratio, monocyte to lymphocyte ratio, mean platelet volume to lymphocyte ratio and platelet count to lymphocyte ratio values were significantly higher in poor glycemic controls in type 2 diabetic patients than good glycemic controlled T2DM ($P < 0.001$) (Table 3). However, absolute lymphocyte count, red blood cells, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration level were significantly lower in type 2 diabetic patients with poor glycemic control than good glycemic controlled T2DM and healthy controls ($p < 0.001$) (Table 3).

Determination of Hematological Parameters and Hemogram-Derived Novel Markers Among T2DM with Microvascular Complication and T2DM Without Microvascular Complication Participants

The Mann–Whitney test showed that there was no significant difference between type 2 diabetics with microvascular complication and those without complication in diabetic patients in terms of red blood cells width standard deviation,

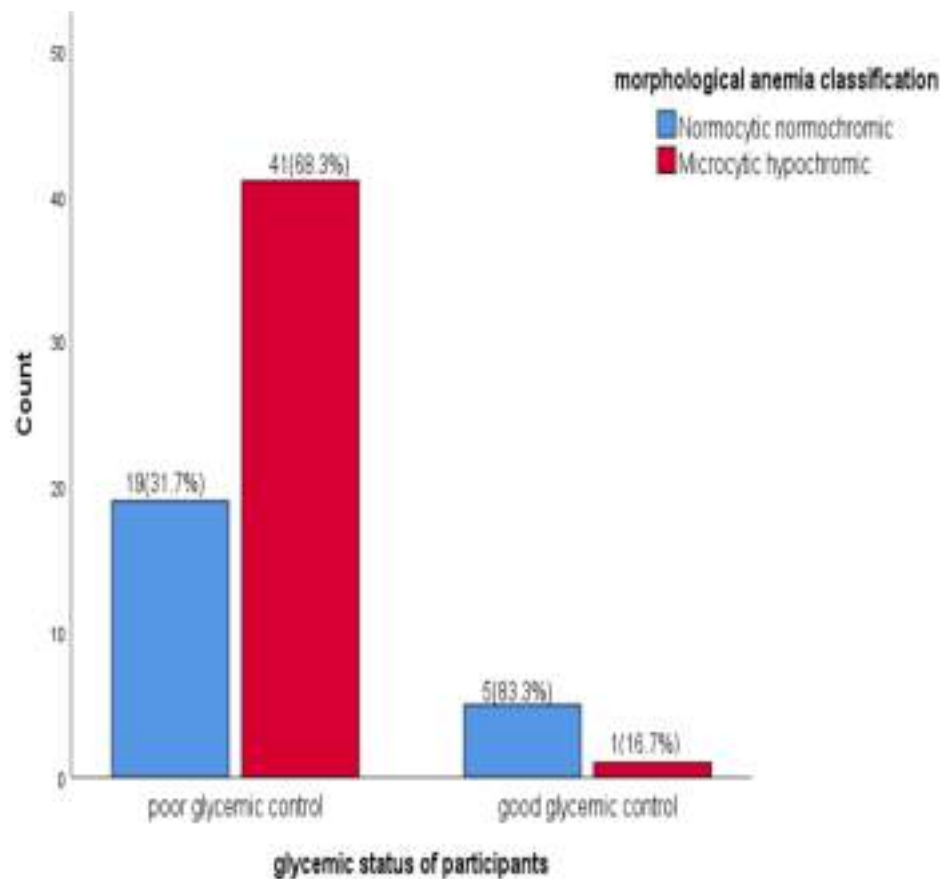


Figure 2 Distribution of morphological type of anemia among type 2 diabetic patients attending Bishoftu Hospital, Oromia, central Ethiopia, from June 15 to August 12, 2022.

absolute eosinophils count, absolute basophils count, Immunoglobulin levels and platelet count to lymphocyte count ratio ($p > 0.05$) (Table 4).

On the other hand, the Mann–Whitney comparison test also showed that the red blood cell width coefficient variation, total white blood cell count, absolute neutrophils count, mean platelet volume, absolute monocyte, neutrophils to

Table 3 Kruskal–Wallis and Post Hoc Pairwise Comparisons of Hematological Parameters and Hemogram Derived Novel Markers Between Poor Glycemic Controlled T2DM, Good Glycemic Controlled T2DM, and Healthy Controls at Bishoftu Hospital, Central Ethiopia, from June 15 to August 12, 2022 (n=261)

Hematological Parameters and Hemogram-Derived Markers	Uncontrolled T2DM n(87)	Controlled T2DM n(87)	Healthy Control n(87)	Kruskal–Wallis Test		Post Hoc Test (Bonferroni Test)	
				P-value	P*	P**	P***
PLT ($10^3/\mu\text{L}$)	188(159–350)	210(162–251)	208(170–232)	0.518	————	————	————
MPV (fl)	12.8(10.8–14.8)	10.7(10–11.2)	9.9(9.6–10.9)	<0.001	0.01	<0.001	<0.001
RBC ($10^6/\mu\text{L}$)	3.38(2.64–4.2)	4.41(4.1–4.8)	4.43(4.2–4.9)	<0.001	0.112	<0.001	<0.001
HGB (g/dl)	9.8(8.2–12.8)	13.8(13.3–14.3)	13.3(12.1–14.7)	<0.001	0.501	<0.001	<0.001
HCT (%)	29.8(26.6–36.8)	38.0(35.2–41.9)	39.4(35.3–44.2)	<0.001	0.366	<0.001	<0.001

(Continued)

Table 3 (Continued).

Hematological Parameters and Hemogram-Derived Markers	Uncontrolled T2DM n(87)	Controlled T2DM n(87)	Healthy Control n(87)		Kruskal-Wallis Test	Post Hoc Test (Bonferroni Test)	
				P-value	P*	P**	P***
MCV (fl)	74.7(66.7–84.0)	87.7(82.6–91.0)	88.7(86–91.9)	<0.001	0.186	<0.001	<0.001
MCH (pg)	29.1(27.3–30.2)	30.4(29.1–31.4)	30.5(28.7–31.4)	<0.001	1.0	<0.001	<0.001
MCHC (g/dl)	32.(25.4–33.7)	33.6(33.1–34.3)	33.6(33.2–34.3)	<0.001	0.111	<0.001	<0.001
RDW-SD (fl)	42.7(40.8–47.3)	41.2(40.1–44.0)	42.1(40.2–45.3)	0.085	————	————	————
RDW-CV (%)	12.8(12.4–14.3)	12.5(12–13.9)	12.7(12.3–14.3)	0.014	0.117	0.014	1.0
WBC	15.9(13.8–17.4)	10.3(6.4–11.7)	7.8(6–10.5)	<0.001	0.147	<0.001	<0.001
NEUT	10.5(8.9–12.2)	5.9(4.1–8.3)	5(3.6–7.9)	<0.001	0.596	<0.001	<0.001
LYMPH	1.4(1.2–1.8)	1.8(1.3–2.3)	1.81(1.3–2.3)	<0.001	1.0	0.001	0.001
MONO	3.3(2.1–4.5)	0.62(0.41–1.71)	0.5(0.4–0.7)	<0.001	0.94	<0.001	<0.001
EO	0.1(0.04–0.2)	0.1(0.04–0.2)	0.11(0.04–0.27)	0.29	————	————	————
BASO	0.3(0.02–0.04)	0.03(0.02–0.04)	0.03(0.02–0.04)	0.997	————	————	————
IG	0.02(0.01–0.04)	0.02(0.01–0.04)	0.02(0.01–0.04)	0.578	————	————	————
NLR	6.9(5.1–10.1)	3.1(1.9–5.2)	2.6(1.8–4.5)	<0.001	0.822	<0.001	<0.001
MLR	2.3(1.1–3.3)	0.3(0.2–1.0)	0.24(0.2–0.43)	<0.001	0.98	<0.001	<0.001
RPR	0.07(0.04–0.09)	0.6(0.05–0.08)	0.07(0.06–0.08)	0.333	————	————	————
MPVLR	8.9(7.1–12.6)	6.1(4.4–8.7)	5.5(4.2–8.1)	<0.001	1.0	<0.001	0.001
PLR	150(103–267)	111(81–165)	106(76–139)	<0.001	1.0	<0.001	0.001

Note: Data are expressed as median (IQR). The p-value was significant at $p < 0.05$; P*-stands for comparison of good glycemic controlled T2DM versus healthy control group; P** stands for comparison of good glycemic controlled T2DM versus poor controlled T2DM; P*** stands for comparison of poor controlled T2DM versus healthy control group.

Table 4 Comparisons of Hematological Parameters and Hemogram Derived Novel Markers Between Type 2 Diabetic Patients with Complications and Without Complications Attending Bishoftu Hospital, Central Ethiopia, from June 15 to August 12, 2022 (n=87)

Hematological Parameters and Hemogram Derived Markers	Microvascular Complicated T2DM Media (IQR) (n=45)	Microvascular Uncomplicated T2DM Media (IQR) (n=42)	P-value
PLT ($10^3/\mu\text{L}$)	169(142–187)	210(172.3–258.5)	<0.001
MPV (fl)	13.9(12.9–15.1)	10.6(9.8–11.4)	<0.001
RBC ($10^6/\mu\text{L}$)	2.95(2.41–4.1)	4.3(3.4–4.8)	<0.001
HGB (g/dl)	9.4(7.9–11.9)	13.4(12.1–14.2)	<0.001
HCT (%)	27.4(25.3–35.3)	37.3(33.7–41.9)	<0.001
MCV (fl)	69.0(64.0–86.9)	87.6(81.0–91.0)	<0.001
MCH (pg)	29.0(28.1–29.9)	30.2(28.7–31.2)	0.024
MCHC (g/dl)	30.5(24.4–33.6)	33.6(32.8–34.3)	0.001

(Continued)

Table 4 (Continued).

Hematological Parameters and Hemogram Derived Markers	Microvascular Complicated T2DM Media (IQR) (n=45)	Microvascular Uncomplicated T2DM Media (IQR) (n=42)	P-value
RDW-SD (fl)	43.1(40.9–48.3)	41.9(40.2–44.5)	0.127
RDW-CV (%)	13.2(12.5–14.6)	12.6(12.2–14.1)	0.004
WBC	15.9(13.2–17.4)	10.3(6.4–13.7)	<0.001
NEUT	10.5(8.6–12.2)	6.6(4.1–9.1)	<0.001
LYMPH	1.4(1.1–1.8)	1.8(1.2–2.2)	0.009
MONO	2.7(0.7–4.3)	0.65(0.42–2.23)	<0.001
EO	0.1(0.04–0.17)	0.1(0.04–0.2)	1.0
BASO	0.03(0.02–0.04)	0.03(0.02–0.04)	0.173
IG	0.02(0.01–0.04)	0.02(0.01–0.04)	0.768
NLR	6.4(4.9–10.1)	3.5(1.9–5.9)	<0.001
MLR	1.7(0.6–2.8)	0.3(0.2–1.2)	<0.001
RPR	0.08(0.07–0.09)	0.6(0.05–0.08)	<0.001
MPVLR	9.0(7.3–13.5)	6.2(4.6–8.8)	<0.001
PLR	121(90–150)	116(88–191)	0.048

Note: Data are expressed as median (IQR).

Abbreviations: MPV - Mean Platelets Volume, MLR - monocyte to lymphocyte ratio, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, MCV - mean corpuscular volume, MPVLR - Mean platelet volume to lymphocyte ratio, NLR - neutrophils to lymphocyte ratio, RDW - red blood cells width, RPR - red blood cells width to platelet count ratio, PCT - Platelets-crit, PDW - Platelets Distribution Width, PLT - Platelets, P-LCR - Platelet Large Cell Ratio, RBC - Red Blood cells, WBC - White Blood Cell.

lymphocyte ratio, monocyte to lymphocyte ratio, mean platelet volume to lymphocyte ratio values were significantly higher in microvascular complicated type 2 diabetic patients than in type 2 diabetes mellitus without complications ($p<0.001$) (Table 4).

In addition, platelet count, absolute lymphocyte count, red blood cells, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration level were significantly lower in type 2 diabetic patients with complications than without complications ($p<0.001$) (Table 4).

Determination of Predictive Values of Hemogram-Derived Novel Markers as Indicators for Poor Glucose Regulation in a Diabetic Patient by ROC Analysis

A ROC analysis was performed to determine the ability of the hemogram novel markers as predictors of poor glycemic control in type 2 diabetic patients. Neutrophils to lymphocyte ratio, Monocyte to lymphocyte ratio, Mean platelet volume to lymphocyte ratio, and Platelet count to lymphocyte ratio have been identified as novel markers for poor glycemic levels in type 2 diabetic patients. However, the Red blood cell width to platelet count ratio failed to discriminate the poor status of glucose regulation from the good status of glucose regulation in type 2 diabetic patients (Table 5 and Figure 3).

Determination of Prognostic Values of Hemogram-Derived Novel Markers as Indicators for Microvascular Complication in a Diabetic Patient by ROC Analysis

A ROC analysis was performed to determine the ability of the hemogram novel markers as forecasters of microvascular complications status in type 2 diabetic patients. Thus, Neutrophils to lymphocyte ratio, Monocyte to lymphocyte ratio, red blood cell width to platelet count ratio, and Mean platelet volume to lymphocyte ratio values were identified as new

Table 5 Determination of Predictive Values of Hemogram-Derived Novel Markers for Poor Glucoregulation of T2DM Patients Attending Bishoftu Hospital, from June 15 to August 12, 2022 (n=174)

Hemogram-Derived Novel Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cut-Off value	AUC	95% CI	P-value
NLR	82%	74%	75.9%	80.4%	≥ 4.7	0.800	(0.732,0.867)	<0.001
MLR	75%	80%	78.9%	76.1%	≥ 1.21	0.831	(0.770,0.892)	<0.001
RPR	53%	64%	59.6%	77.1%	≥ 0.07	0.501	(0.412,0.590)	0.984
MPVLR	84%	61%	79.2%	68.3%	≥ 6.74	0.752	(0.679,0.824)	<0.001
PLR	66%	61%	62.9%	64.2%	≥ 120.4	0.657	(0.576,0.738)	<0.001

Note: Data are expressed as frequency, or percentage.

Abbreviations: PPV - positive predictive value, NPV - negative predictive value, AUC - area under a curve, 95% CI - at 95% confidence interval.

markers of microvascular complications in type 2 diabetic patients. Platelet counts to lymphocyte ratio failed to discriminate the microvascular complications status from non-complications status in type 2 diabetic patients (Table 6 and Figure 4).

Discussion

Among hematological disorders, anemia is the most common blood disorder. Due to chronic inflammation, a leukocyte count increment plays a pivotal role in the development of diabetic macro- and microvascular complications. Other vascular complications cause the alteration of platelet morphology and functions.^{9,11} Determination of hematological parameters and hemogram-derived markers have received attention in the management of diabetic patients because they are used to ascertain diabetic poor glycemic and vascular complications status in a simple, rapid, and inexpensive manner.^{11,31–37}

In our study about 37.9% of type 2 diabetic participants experienced anemia. In diabetic patients anemia is common due to oxidative stress; increased erythrocyte mechanical fragility; Impact of hypoglycemic drugs; relative erythropoietin (EPO) deficiency, shortened erythrocyte lifespan by the coexistence of micro- and macro-angiopathies.^{9,11}

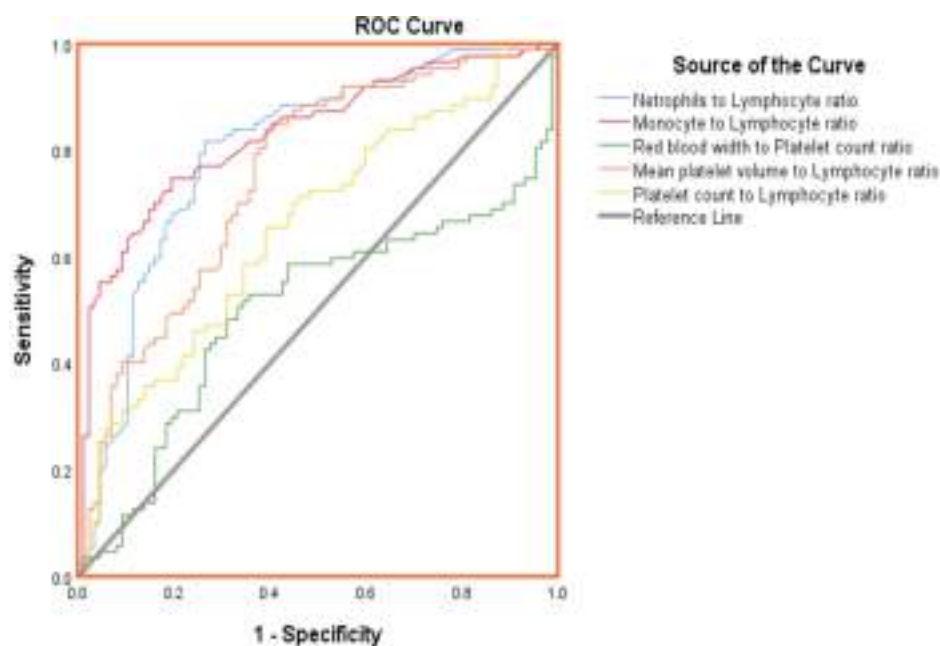
**Figure 3** Graph of Receiver operating characteristic curve analysis for hemogram-derived novel markers as a predictor of poor glucoregulation in type 2 diabetic patients attending Bishoftu General Hospital, from June 15 to August 12, 2022.

Table 6 Determination of Projecting Capabilities of Hemogram-Derived Novel Markers for Microvascular Complication in T2DM Patients Attending Bishoftu Hospital, from June 15 to August 12, 2022 (n=87)

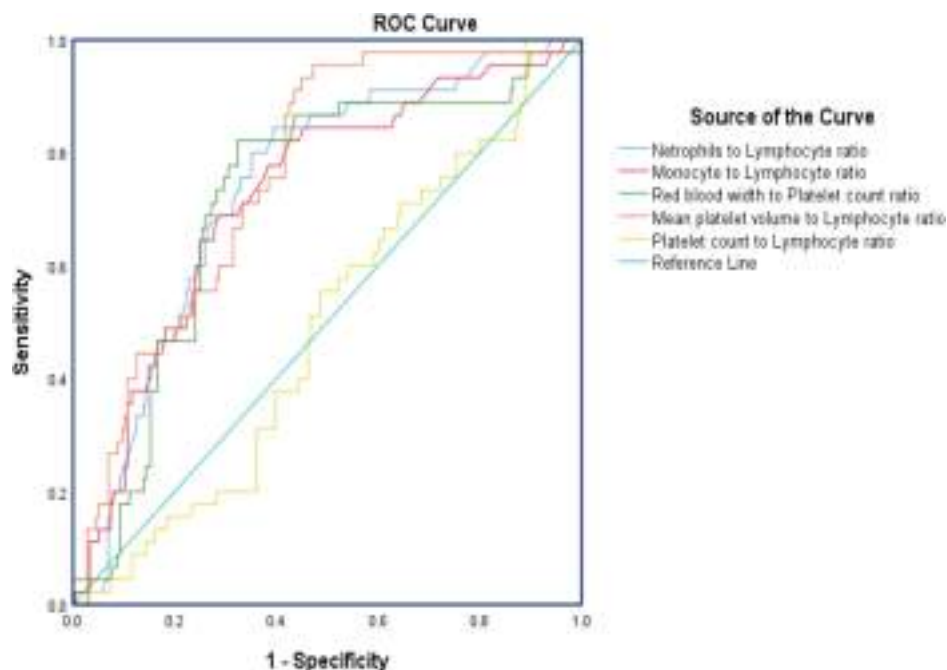
Hemogram-Derived Novel Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cut-Off value	AUC	95% CI	P-value
NLR	65%	80%	76.5%	69.6%	≥ 5.7	0.738	(0.663,0.812)	<0.001
MLR	69%	72%	71.1%	69.9%	≥ 0.93	0.724	(0.644,0.805)	<0.001
RPR	82%	68%	80.4%	79.1%	≥ 0.07	0.718	(0.636,0.799)	<0.001
MPVLR	91%	56%	67.4%	86.2%	≥ 6.80	0.759	(0.691,0.828)	<0.001
PLR	71%	36%	52.6%	55.4%	≥ 101.7	0.044	(0.403,0.574)	0.805

Note: Data are expressed as frequency, or percentage.

Abbreviations: PPV - positive predictive value, NPV - negative predictive value, AUC - area under a curve, 95% CI - at 95% confidence interval.

Our study showed lower anemia prevalence than the report of the study done in Karachi.³⁸ This disagreement might be due to differences in the age of the current and previous study population. For example, in contrast to the current study which included adult participants between 18–65 years old, the study conducted in Karachi included those who were 18–85 years old. Reduced renal function, inflammation, bone marrow suppression, and malnutrition are more probable as people become older.³⁹ Likewise, a study conducted in Karachi included 18.4% of participants with a history of blood loss and 5.4% of patients were strict vegetarians. Those two key factors might cause the difference in anemia prevalence. Our study revealed higher anemia proportion when compared with studies done in other parts of Ethiopia.^{40,41} This inconsistency might be attributed to variations in the sample size and behavioral characteristics of the study subjects. For instance, a study done in Eastern Ethiopia involved 325 and North-East Ethiopia involved 249 study participants. Our study involved only 174 participants to determine anemia prevalence; which might be statistically influenced and gave exaggerated anemia prevalence in the current study.

This study aimed to determine the conjoint morphological characteristics of anemia among T2DM participants. The normocytic normochromic and microcytic blood pictures were the most common morphological blood pictures. It is not

**Figure 4** Graph of Receiver operating characteristic curve analysis for hemogram-derived novel markers as a predictor of microvascular complications in type 2 diabetic patients attending Bishoftu General Hospital, from June 15 to August 12, 2022.

surprising to see a normocytic normochromic blood picture of anemia in this study, as preceding studies conducted in Karachi³⁸ and Iran,⁴² also had comparable findings. As the participants of this study were DM patients, anemia of chronic disease is predictable, which is normocytic normochromic in morphology. However, microcytic hypochromic anemia experienced by DM patients also might be due to nutritional deficiencies and poly-pharmacy frequently being used.⁴³

This study result suggests that there was no significant difference in PLT count, red blood cell width, absolute eosinophil count, basophils count, immunoglobulin level, and red blood cell width to platelet count ratio between diabetics with and without poor glycemic control and healthy control groups. This was in line with the conclusions of studies conducted in Turkey.^{44–46} Furthermore, there was no significant difference in terms of absolute eosinophil count, absolute basophils count, immunoglobulin level, and red blood cell width standard deviation between type 2 diabetic patients with microvascular and without microvascular complications. The reports from studies conducted in India,⁴⁷ Indonesia,³² and China²¹ are concordant with our study result.

The results of the current study revealed that elevated values of total WBC, absolute neutrophils count, absolute monocyte count, mean platelet volume, neutrophils to lymphocyte ratio, monocyte to lymphocyte ratio, mean platelet volume to lymphocyte ratio, and platelet count to lymphocyte ratio in poor glycemic control type 2 diabetic compared with good glycemic control and healthy control group. The inflammatory condition in type 2 diabetic patients leads to increased production of monocytes and neutrophils, which enhance the increase of their hemogram derivative.⁴⁸ Our findings were consistent with studies conducted in Turkey.^{33,44} On the other hand, a study conducted in Indonesia⁴⁹ found contradictory results of absolute Neutrophil count, Monocyte count, and lymphocyte count with our findings. This discrepancy may be due to several factors such as body mass index, lipid profile, blood pressure controlling mode, and duration of suffering by DM of current and previous study participants since not controlling these risk factors causes rise of WBC, monocyte, neutrophil and their derivative in diabetic patients.

In our study, the values of red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and absolute lymphocyte count were lower among poor glycemic control type 2 diabetics than good glycemic control and healthy control group. Hyperglycemia has a direct relationship with the development of an inflammatory condition showed by the increased expression of proinflammatory cytokines such as IL-6, TNF- α , and NF κ B. By increasing especially IL-6, an anti-erythropoietic effect occurs, since this cytokine changes the sensitivity of progenitors to erythropoietin and also increases apoptosis of immature erythrocytes initiating a decrease further in the number of erythrocytes and consequently causing a reduction of circulating hemoglobin.⁵⁰ In T2DM patients, the lymphocyte is lower due to insufficiency of lymphocyte proliferation following decreased expression of interleukin-2 receptors (IL-2) because of Clusters of Differentiation Antigen 25 (CD25) deficiency.⁴⁸ Studies conducted in India,⁵¹ Bangladesh,³¹ and Indonesia,⁵² also support our findings.

The values of mean platelet volume, red blood cells width coefficient of variation, total WBC count, absolute neutrophils count, absolute monocyte count, neutrophils to lymphocyte ratio, monocyte to lymphocyte ratio, mean platelet volume to lymphocyte ratio, and red blood cells width to lymphocyte ratio significantly increased among type 2 diabetics with microvascular complications compared with type 2 diabetics without complications. Reports similar to our findings come from studies conducted in Turkey,^{23,34} Syria,⁵³ China,^{21,37,54,55} and Egypt.⁵⁵ On the other hand, a study conducted in China²¹ found inconsistent results to our findings. The possible reason for this contradiction might be due to the differences in the diastolic and systolic blood pressure levels in the current study and the study done in China.²¹ Since these blood pressure parameters are increased in our study subjects could amplify the inflammation condition in the participants increasing inflammatory indicators parameters.

The values of platelet count, red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and absolute lymphocyte count significantly decreased among type 2 diabetics with microvascular complications than type 2 diabetics without complications. The exact mechanisms of lower RBC parameters in patients with microvascular complications is not clear.⁵⁶ However, the effect of DM on tissues responsible for the synthesis of erythropoietin reduces kidney response to hypoxia. Reduction in androgenic hormone levels in DM attenuates stem cells in the bone marrow and reduces erythropoietin synthesis in the kidney. Reticulocytes also develop resistance in their response to increased erythropoietin.⁵⁷

Studies conducted in India,^{47,58} China,^{21,51} Syria,⁵³ Iran,⁴² and Italy⁵⁹ also support our findings. However, a study done in India⁵⁸ revealed only Hgb, MCV, and MCHC values were significantly decreased in cases as compared with the control group; and a study done in Saudi Arabia³² came only with lower RBC count, Hgb, Hct values in cases compared with controls. The possible reason for inconsistency of our study findings with studies done in India and Saudi Arabia could be due to differences in the onset duration of complications. Since the longer duration of complication onset might cause decrement of RBC parameters.

According to our study, the results of the Receiver Operating Characteristic Curve (ROC) analysis showed that NLR, MLR, MPVLR, and PLR can distinguish poor glycemic control status from good control in type 2 diabetic patients. However, the RPR value failed to distinguish the poor glycemic status from the good glycemic status in diabetic patients. Based on their AUC values, the NLR and MLR were identified as excellent predictors of poor glycemic status; while MPVLR and PLR values were good forecasters of poor glycemic control in diabetic patients. The values of NLR, PLR, and MLR are potential inflammatory biomarkers for tumors, cardiovascular disease, ankylosing spondylitis, rheumatoid arthritis, and inflammatory bowel disease. Currently, there is evidence that connects them to the progression of DM and its complications.^{12–14,17–22}

Regarding our study, the results of Receiver Operating Characteristic curve (ROC) analysis showed that the NLR, MLR, MPVLR, and RPR values were identified as novel markers of microvascular complications in type 2 diabetic patients. Although numerous tests are used, as a prognostic tool for diabetic-triggered microvascular complications, the complete blood count with differential is extensively used, either alone or in combination with other tests and hemogram-derived markers is endorsed. However, the PLR value failed to distinguish the microvascular complication status in diabetic patients. Based on their AUC values, the NLR, MLR, MPVLR, and RPR values were identified as good prognosticators of microvascular complication status in diabetic patients. The AUC, cutoff value, specificity, and sensitivity values of NLR were greater in our study than in studies done in China³² and in India.³⁶ However, a study conducted in China⁵¹ reported a higher sensitivity value than ours. The possible reason for these contradictory values could be due to inadequate glycemic control despite the use of different treatment medicines in our study participants.

In our study, the AUC, cutoff value, and sensitivity values of MPVLR were greater compared with the result of a study done in Turkey,²³ while the specificity value of MPVLR was lower in ours. This inconsistency may be due to the effects of confounding factors and differences in sample size. The sensitivity, specificity, and AUC values of MLR in our study were lower as compared with a study done in China.⁵⁴ However, our study comes up with a greater cutoff value. In addition, the sensitivity, specificity, AUC, and cutoff values of MLR in our study were significantly higher when compared with the results of a study conducted in China.²² The disagreement may be due to the effects of confounding factors and differences in lifestyle of the previous and present study participants. Although lifestyle dynamics might be an unmeasured confounding factor for this connotation, sex hormones have many potential effects on several organs with cardiovascular and immune purposes.⁶⁰

The RPR with a sensitivity of 82%, specificity of 68%, PPV of 80.4%, NPV of 79.1%, AUC 0.718, and cutoff values of 0.718 were identified as predictors of poor microvascular complications. In our study, the AUC, specificity, and NPV values were significantly lower than in a study done in Turkey.³⁵ While, in our study PPV, cutoff values, and sensitivity values were significantly higher than a study done on Diagnostic Possibilities for Early Onset Neonatal Sepsis: Red Cell Distribution Width to Platelet Ratio study done in Turkey.³⁵ In comparison to inflammatory cytokines, the calculation approaches for the NLR, PLR, MLR, MPVLR, and RPR are fairly straightforward, as they can be attained through peripheral blood examination, which is cheap, appropriate, and simple to explain.

The Strength of This Study

This study is a comprehensive study to evaluate nearly all hematological parameters in various conditions of type 2 diabetic patients and it can give clues on the purpose of hematological parameters in the management and control of diabetic patients. In addition, this study also assesses the level of different hemogram-derived novel markers in the different health statuses of type 2 diabetic patients and this may create an outline for other researchers. Also,

a morphological study of blood cells was done for the morphological characterization of anemia and to check the hematological analyzer output of complete blood count.

Limitations of This Study

This study used a cross-sectional study design that limits the ability to identify causal relationships between variables. Also, it uses a small sample size and is conducted at a single hospital. In addition, an assessment of the coagulation profile was not done, and plasma glucose was used instead of HgbA1c due to limitations of the HgbA1c test and coagulation profile assessment set up in the hospital.

Conclusion

We conclude our study by saying that anemia is common in diabetes mellitus, particularly in patients with poor glycemic control and microvascular complications. The values of total WBC count, absolute neutrophils count, absolute monocyte count, NLR, MLR, MPVLR, and PLR values were significantly higher in poor glycemic control T2DM; and also NLR, MLR, MPVLR, and RPR values were significantly higher in DM patients with microvascular complications. On the other hand, RBC count, HGB, HCT, MCV, MCH, and MCHC levels and the absolute lymphocyte count values were significantly lower in T2DM with poor glycemic control and microvascular complications.

Correspondingly, the ROC analysis showed that NLR, MLR, MPVLR, and PLR were significantly identified as potential prognosticators for poor glycemic control. While, NLR, MLR, MPVLR, and RPR were significantly identified as possible forecasters for microvascular complication status in diabetic patients. These hemogram-derived markers are more useful than other cell parameters; because they have absolute value counts and show good constancy even when pathological, physiological, and physical factors of the WBC count fluctuate. Therefore, routine screening of hematological parameters, and calculation and use of hemogram-derived markers for monitoring and management of different altered health status of DM is very important in the improvement of patients' quality of life.

Abbreviations

AIDS, Acquired Immune Deficiency Syndrome; ADA, American Diabetes Association; ART, Ant-retrovirus treatment; BP, Blood Pressure; BMI, Body Mass Index; CBC, Complete Blood Count; DM, Diabetes Mellitus, K2-EDTA, Dipotassium Ethylene-Diamine Tetra-acetic Acid; FBS, Fasting Blood Glucose; HC, Hip-Circumference; HIV, Human Immune Virus; HBV/C, Hepatitis virus B or C; IDF, International Diabetes Federation; IQR, Interquartile Range; MPV, Mean Platelets Volume; MLR, monocyte to lymphocyte ratio; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPVLR, Mean platelet volume to lymphocyte ratio; NLR, neutrophils to lymphocyte ratio; RDW, red blood cells width; RPR, red blood cells width to platelet count ratio; ORS, Oromia Regional State; PCT, Platelets crit; PDW, Platelets Distribution Width; PLT, Platelets; P, LCR-Platelet Large Cell Ratio; ROC, Receiver Operating Characteristic curve; RBC, Red Blood cells; SBP, Systolic Blood Pressure; SPSS, Statistical Package for Social Sciences, T2DM, Type 2 Diabetes Mellitus; US\$, United State Dollar; WBC, White Blood Cell; WC, Waist Circumference; WHO, World Health Organization; WHR, Waist to Hip Ratio.

Data Sharing Statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics and Information Consent to Participate

The study was conducted following the Declaration of Helsinki and an approved ethical clearance was obtained from the Institutional Review Board of the Jimma University Institute of Health under Ref.No.IHPPGJ/84412 at date:30/05/2022. A supplementary letter, with Ref.No.BEFO10339/2022, was also obtained from the Oromia Regional Office, Department of Research Review Board, and submitted to the Bishoftu General Hospital administration office and was directed to the medical laboratory head and chronic clinic head offices. It was then sent to both headquarters along with a letter of support from the School of Medical Laboratory Sciences, Jimma University.

After obtaining permission from the Hospital administrator, the head of the Chronic Clinic care unit, and the head of the medical laboratory, a clear explanation of the study's objectives, procedures, benefits, possible risks, and the participant's right to voluntarily participate, written informed consent was obtained from each study subject. Codes were used instead of participants' names to protect the confidentiality of obtained data, and unauthorized person access to the collected data was prohibited.

Acknowledgments

All authors would like to thank Jimma University, Bishoftu General Hospital staff members, all data collectors, supervisors, study participants, and questionnaire translators for their assistance in conducting this study.

Author Contributions

All authors made a significant contribution to the work reported in the conception, study design, execution, acquisition, analysis, and interpretation of data; took part in the drafting, revising, or critical reviewing of the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors declare no conflicts of interest in this study.

References

1. World Health Organization. *Geneva: Classification of Diabetes Mellitus*. World Health Organization. 4–28. 2019.
2. American association Diabetes. Classification and diagnosis of diabetes: standards of medical care in diabetes. *J Diabetes Care*. 2019;42(1):13–28. doi:10.2337/dc19-S002
3. World Health Organization (WHO). *Geneva: Diabetes Key Facts Overview*. World Health Organization. 1–5. 2021.
4. International Diabetes Federation (IDF). *IDF Diabetes Atlas*. 10th ed. International Diabetes Federation (IDF); 2021: 9–65.
5. Zeru M, Tesfa E, Mitiku A, et al. Prevalence and risk factors of type-2 diabetes mellitus in Ethiopia: systematic review and meta-analysis. *Sci Rep*. 2021;11(1):1–15. doi:10.1038/s41598-021-01256-9
6. Victoria H, Reimar W, Ole H, et al. Diabetes in Sub Saharan Africa: epidemiology and public health implications. *BMC Public Health*. 2011;11:1471–2458.
7. Ling W, Huang Y, Huang Y, et al. Global trend of diabetes mortality attributed to vascular complications, 2000–2016. *J Cardiovasc Diabetol*. 2020;19(182):2–12.
8. Bandeira Sde M, da Fonseca LJS, Guedes Gda S, et al. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. *Int J Mol Sci*. 2013;14(2):3265–3284. doi:10.3390/ijms14023265
9. Papatheodorou K, Banach M, Bekiari E, et al. Complications of diabetes. *J Diabetes Res*. 2018;30(8):61–67.
10. Iglay K, Hannachi H, Joseph Howie P, et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. *Curr Med Res Opin*. 2016;32(7):1243–1252. doi:10.1185/03007995.2016.1168291
11. Skyler JS, Bakris GL, Bonifacio E, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis Diabetes. *Am Diabetes Assoc Inc*. 2017;66:1.
12. Orozco-Morales M, Soca-Chafre G, Barrios-Bernal P, et al. Interplay Cancer, between cellular and molecular inflammatory mediators in lung. *Inflamm Mediat Journal*. 2016;34(9):46–108.
13. Marchetti C, Romito A, Musella A, et al. Combined plasma fibrinogen and neutrophil lymphocyte ratio in ovarian cancer prognosis may play a role? *Int J Gynecol Cancer*. 2018;28(5):939–944. doi:10.1097/IGC.0000000000001233
14. Fei X, Xing M, Wo M, et al. Thyroid stimulating hormone and free triiodothyronine are valuable predictors for diabetic nephropathy in patient with type 2 diabetes. *Ann Trans Med*. 2018;6(15):30–35. doi:10.21037/atm.2018.07.07
15. Kraakman MJ, Lee MKS, Al-Sharea A, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J Clin Invest*. 2017;127(6):2133–2147. doi:10.1172/JCI92450
16. Ebrahim H, Fiseha T, Ebrahim Y, et al. Comparison of hematological parameters between type 2 diabetes mellitus patients and healthy controls at Dessie comprehensive specialized hospital, Northeast Ethiopia: comparative cross-sectional study. *PLoS One*. 2022;17(7):e0272145. doi:10.1371/journal.pone.0272145
17. Fu W, Fu H, Ye W, et al. Peripheral blood neutrophil-to-lymphocyte ratio in inflammatory bowel disease and disease activity: a meta-analysis. *Int Immunopharmacol*. 2021;101(10):82–85. doi:10.1016/j.intimp.2021.108235
18. Gemenetzi G, Bagante F, Griffin J, et al. Neutrophil-to-lymphocyte ratio is a predictive marker for invasive malignancy in intraductal papillary mucinous neoplasms of the pancreas:339–345. *Ann Surg*. 2017;266(2):339–345. doi:10.1097/SLA.0000000000001988

19. Patel A, Ravaud A, Motzer R, et al. Neutrophil-to-lymphocyte ratio as a prognostic factor of disease-free survival in postnephrectomy high-risk locoregional renal cell carcinoma: analysis of the S-TRAC trial. *Clin Cancer Res*. 2020;26(18):4863–4868. doi:10.1158/1078-0432.CCR-20-0704
20. Liang T, Chen J, Xu G, et al. Platelet-to-lymphocyte ratio as an independent factor was associated with the severity of ankylosing spondylitis. *Front Immunol*. 2021;12:214–276. doi:10.3389/fimmu.2021.760214
21. Zeng J, Min Chen QF, Feng Q, et al. The platelet-to-lymphocyte ratio predicts diabetic retinopathy in type 2 diabetes mellitus. *Diabetes, Metab Syndr Obes Targets Ther*. 2022;15:3617–3626. doi:10.2147/DMSO.S378284
22. Yue S, Zhang J, Wu J, et al. Use of the monocyte-to-lymphocyte ratio to predict diabetic retinopathy. *Int J Environ Res Public Heal*. 2015;12(8):10009–10019. doi:10.3390/ijerph120810009
23. Kocak MZ, Aktas G, Erkus E, et al. Mean platelet volume to lymphocyte ratio as a novel marker for diabetic nephropathy. *J Coll Physicians Surg Pakistan*. 2018;28(11):844–847. doi:10.29271/jcpsp.2018.11.844
24. Gebreegziabher E, Astawesegn F, Anjulo A, et al. Urban health extension services utilization in bishoftu Town, Oromia Regional State, Central Ethiopia. *BMC Health Serv Res*. 2017;17(1):1–10. doi:10.1186/s12913-016-1943-z
25. Gedamu S, Sendo E, Daba W, Talbott EO. Congenital anomalies and associated factors among newborns in Bishoftu General Hospital, Oromia, Ethiopia: a retrospective study. *J Environ Public Health*. 2021;2021:1–6. doi:10.1155/2021/2426891
26. Biadgo B, Melku M, Abebe S, et al. Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. *J Diabetes Metab Syndr Obes Targets Ther*. 2016;9:91–99. doi:10.2147/DMSO.S97563
27. VanVoorhis CW, Morgan BL. Understanding power and rules of thumb for determining sample sizes. *Tutor Quant Methods Psychol*. 2007;3(2):43–50.
28. Ivanov TD. American Diabetes Association. *Stand Med Care Diabetes-2017*. 2017;6(1):47–63.
29. Centers for Disease Control and Prevention. Laboratory procedure manual for fasting glucose by roche cobas C311. 2017. Available from: https://www.cdc.gov/nchs/data/nhanes/2015-2016/labmethods/GLU_I_MET_C311.pdf. Accessed 27 December 2023.
30. Grigg A Europe, Sysmex XN-550 complete blood count and parameters whole blood-standard operating procedure. 2019;1–25.
31. Jaman MS, Rahman MS, Swarna RR, et al. Diabetes and red blood cell parameters. *Ann Clin Endocrinol Metab*. 2018;2(1):1–9.
32. Alamri BN, Bahabri A, Aldereihim AA, et al. Hyperglycemia effect on red blood cells indices. *Eur Rev Med Pharmacol Sci*. 2019;23(5):2139–2150. doi:10.26355/eurrev_201903_17259
33. Sefil F, Ulutas KT, Dokuyucu R. Investigation of neutrophil lymphocyte ratio and blood glucose regulation in patients with type 2 diabetes mellitus. *J Int Med Res*. 2014;42(2):581–588. doi:10.1177/0300060513516944
34. Ulu SM, Dogan M, Ahsen A, et al. Neutrophil-to-lymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. *DIABETES Technol Ther*. 2013;15(11):943–947. doi:10.1089/dia.2013.0097
35. Karabulut B, Arcagok BC. New diagnostic possibilities for early onset neonatal sepsis: red cell distribution width to platelet ratio. *Fetal Pediatr Pathol*. 2020;39(4):297–306. doi:10.1080/15513815.2019.1661051
36. Dutta S, Qureshi Z, Surana V, et al. Neutrophil-lymphocyte Ratio is a Novel Reliable Predictor of Nephropathy, Retinopathy, and Coronary Artery Disease in Indians with Type-2 Diabetes. *Indian J Endocrinol Metab*. 2017;21(6):865–869.
37. Wang JR, Chen KY, Chi C. Association between neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and diabetic retinopathy. *Diabetol Metab Syndr*. 2020;12:1–10. doi:10.1186/s13098-019-0485-z
38. Shams N, Osmani MH. Newly diagnosed anemia in admitted diabetics, frequency, etiology and associated factors. *J Coll Physicians Surg Pakistan*. 2015;25(4):242–246.
39. Trevest K, Treadway H, Hawkins-van DCG, Bailey AA, Abdelhafiz AH. Prevalence and determinants of anemia in older people with diabetes attending an outpatient clinic: a cross-sectional audit. *Clin Diabetes*. 2014;32(4):158. doi:10.2337/diaclin.32.4.158
40. Tujuba T, Ayele BH, Fage SG, et al. Anemia among adult diabetic patients attending a general hospital in Eastern Ethiopia: diabetes. *Metab Syndr Obes*. 2021;14:467–476. doi:10.2147/DMSO.S289298
41. Taderegew MM, Gebremariam T, Tareke AA, Woldeamanuel GG. Anemia and its associated factors among type 2 diabetes mellitus patients. *J Blood Med*. 2020;11:47–58. doi:10.2147/JBM.S243234
42. Hosseini MS, Rostami Z, Saadat A, et al. Anemia and microvascular complications in patients with type 2 diabetes mellitus. *Nephro Urol Mon*. 2014;6(4):e19976. doi:10.5812/numonthly.19976
43. Thambiah SC, Samsudin IN, George E, et al. Anaemia in type 2 diabetes mellitus (T2DM) patients in Hospital Putrajaya. *Malaysian J Med Heal Sci*. 2015;11(1):49–62.
44. Duman TT, Aktas G, Atak BM, et al. Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. *African Heal Sci*. 2019;19(1):1602–1606. doi:10.4314/ahs.v19i1.35
45. Pujani M, Gahlawat H, Agarwal C, Chauhan V, Singh K, Lukhmana S. Can they serve as biomarkers of glycemic control or development of complications in evaluation of type 2 diabetes mellitus? *Iraqi J Hematol*. 2018;7(2):72–78. doi:10.4103/ijh.ijh_8_18
46. Kadić HS. Mean platelet volume predicts the glycemic control deterioration in diabetes mellitus type 2 patients. *J Med Glas*. 2016;13(1):1–7.
47. Farooqui R, Afsar AI, Afroz IA. Role and significance of hematological parameters in diabetes mellitus. *Ann Pathol Lab Med*. 2019;6(3):159–162. doi:10.21276/apalm.2355
48. Nurlaelatiningsih SS. Correlation between neutrophil- lymphocyte ratio and cardiac autonomic neuropathy in diabetes mellitus type 2 patients. *Int J Integr Heal Sci*. 2017;5(1):15–20.
49. Adnyani PY, Mahartini NN, Herawati S, et al. Comparison of Neutrophil to Lymphocyte Ratio (NLR) and Lymphocyte to Monocyte Ratio (LMR) values in controlled and uncontrolled Type 2 Diabetes Mellitus (T2DM) patient. *Bali Med J*. 2021;10(2):2302–2914. doi:10.15562/bmj.v10i2.2501
50. Angelousi EL, Larger E. Anaemia, a common but often unrecognized risk in diabetic patients: a review. *Diabetes Metab*. 2015;41(1):18–27. doi:10.1016/j.diabet.2014.06.001
51. Xu T, Weng Z, Chu P, et al. The relationship between neutrophil-to-lymphocyte ratio and diabetic peripheral neuropathy in Type 2 diabetes mellitus. *Medicine*. 2017;96(45):1–6.
52. Sari NKP, WandelN I, Ketut Suega IK, et al. The relationship between hepcidin and anemia in controlled and uncontrolled Type-2 Diabetes Mellitus (T2DM) patients. *Indones J Biomed Sci*. 2021;15(2):140–144. doi:10.15562/ijbs.v15i2.361
53. Jaaban M, Zetoune AB, Hesenow S, et al. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as novel risk markers for diabetic nephropathy in patients with type 2 diabetes. *Heliyon*. 2021;7(7):e07564. doi:10.1016/j.heliyon.2021.e07564

54. Huang Q, Wu WM, Kumaran EAP, et al. Monocyte-lymphocyte ratio is a valuable predictor for diabetic nephropathy in patients with type 2 diabetes. *Medicine*. 2020;18(1):1–7. doi:10.1186/s12916-019-1443-1
55. Moursy EY, Megallaa MH, Mouftah RF, et al. Relationship Between Neutrophil-Lymphocyte Ratio and Microvascular Complications. *Am J Intern Med*. 2015;3(6):250–255.
56. King DE, Mainous A, Buchanan PW, Pearson WS. C-reactive protein and glycemic control in adults with diabetes. *Diabetes Care*. 2013;26(5):1535–1539. doi:10.2337/diacare.26.5.1535
57. Craig KJ, Williams JD, Riley SG, Smith H, Owens WD. Anemia and diabetes in the absence of nephropathy. *Diabetes Care*. 2015;28(5):1118–1123. doi:10.2337/diacare.28.5.1118
58. Samuel R, Tejaswi N, Kumar PN, et al. Clinical significance of screening for anaemia in diabetic patients. *Int J Pharm Sci Rev Res*. 2018;48(2):20–24.
59. Lippi G, Mercadanti M, Aloe R, et al. Erythrocyte mechanical fragility is increased in patients with type 2 diabetes. *Eur J Intern Med*. 2012;23(2):150–153. doi:10.1016/j.ejim.2011.11.004
60. Cohen J, Vincent J, Adhikari N, Machado F, Angus CT. Sepsis: a roadmap for future research. *Lancet Infect Dis*. 2018;10(2):8.

Publish your work in this journal

The Journal of Blood Medicine is an international, peer-reviewed, open access, online journal publishing laboratory, experimental and clinical aspects of all aspect pertaining to blood based medicine including but not limited to: Transfusion Medicine; Blood collection, Donor issues, Transmittable diseases, and Blood banking logistics; Immunohematology; Artificial and alternative blood based therapeutics; Hematology; Biotechnology/nanotechnology of blood related medicine; Legal aspects of blood medicine; Historical perspectives. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/journal-of-blood-medicine-journal>