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CASE REPORT

Type 1 Gaucher's Disease. A Rare Genetic Lipid Metabolic Disorder Whose Diagnosis Was Concealed by Recurrent Malaria Infections in a 12-Year-Old Girl

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Introduction: Gaucher disease is a rare autosomal recessive lysosomal storage disease with unknown prevalence in Africa and no record of the disease exists in Uganda.

Case Presentation: We report a case of a 12-year-old female, the last born of 6 from a family with no known familial disease who presented with non-neuronopathic Gaucher disease and superimposed malaria. The disease was initially misdiagnosed as hyperreactive malarial splenomegaly but was subsequently confirmed by examination of the bone marrow smear and core. The disease was managed supportively and splenectomy was done due to worsening hematological parameters. She currently takes morphine for bone pains in addition to physiotherapy.

Conclusion: Always HMS is a common complication in malaria endemic areas, other causes of hepatosplenomegaly need to be excluded before the diagnosis is made. Diagnosis and treatment of patients with rare conditions like GD is still a challenge in developing countries. Although splenectomy is indicated in GD, it should only be done when it is absolutely necessary.

Keywords: type 1 Gaucher's disease, case report, Uganda

Introduction

Gaucher disease (GD) is an autosomal recessive lysosomal storage disease (LSD), caused by over 300 mutations in the GBA1 gene located on the long arm of chromosome 1, region 2 band 1.[1,2] This causes a deficiency of a key lysosomal enzyme called β-glucosidase (also known as glucocerebrosidase) in the leukocytes. This enzyme is critical in the metabolic conversion of glucosylceramides (sugar-containing fat) to glucose and ceramide. Because of its deficiency, glucosylceramides accumulate in the lysosomes (digestive machinery of macrophages), transforming the macrophages into Gaucher cells that characterize Gaucher disease.[3] This material/substrate (glucosylcerebroside) is continuously produced by the body but the lysosomes cannot break it down, it gets "stored" in the macrophages, hence its name, LSD. Therefore, these transformed macrophages accumulate in the body's organs like bone marrow, spleen, liver, and nerves, impairing these organs' functions.[4] GD has no sex predilection and is the second most common lipid storage disease after Fabry disease.[5]

It is a rare disease with a prevalence of 1 in 100,000 and an incidence of 1 to 60,000 births in the general population.[3] The incidence drastically rises to about 1 in 450 births among Ashkenazi Jews.[6] In Africa, data is limited, however, a number of cases have been reported by several authors, from South Africa, Morocco, Mali, and Kenya.[2,6–8] In Uganda, data about the condition is lacking. To the best of our knowledge, this is the first case of Gaucher disease to be reported in the country.

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The disease can present in either of the 3 forms. The non-neuronopathic form (GD type 1) and the neuronopathic form (GD type 2 and 3).[3,9] Type 1 disease is characterized by the absence of neurological involvement and may present with pancytopenia, organomegaly (hepatosplenomegaly), skeletal lesions, and lung and renal involvement. It is the most common form of the disease among the Jews. Type 2 is characterized by central nervous system involvement and is rapidly fatal. It is more common among infants and newborns and is also known as the acute neuropathic form. Type 3 disease also causes central nervous system involvement; however, it takes a more chronic form and also causes hematological and skeletal involvement. Sufferers can live up to 40 years and the disease is known as the chronic neuropathic form.[1,5] Types 2 and 3 are more common among the non-European population.[1]

Case Presentation

A 12-year-old Ugandan female, referred from a peripheral health center with 2 years history of on-and-off spontaneous progressively worsening epistaxis, associated with an 8/12 history of progressive abdominal distension, and mild pain. The bleeding is preceded by a sharp frontal throbbing headache. Two weeks prior to admission, epistaxis worsened with up to 4 episodes per day with an increase in abdominal distension and pain. In the same period, she developed on-and-off low-grade fevers, associated with severe headaches, palpitations, dizziness, and general malaise. She had had 3 admissions last year for which she was managed for malaria, with no blood transfusion. She is the last born of 6 and had no family history of a similar condition.

Examination revealed moderate anemia, mild jaundiced, and nontender mild submandibular lymphadenopathy. Was pyrexic with an axillary temperature of 38.4°C, tachycardic at 120 beats per minute. Other vitals were unremarkable. She had a non-tender moderately distended abdomen, with a massive hepatosplenomegaly (spleen 8cm below the coastal margin and liver 12 cm below the coastal margin) (see Figure 1). Other organ systems were unremarkable.

Investigations revealed pancytopenia (Hemoglobin of 5.9g/dl, White cell count of 2100/µL, platelets of 76,000/µL) (See Table 1 for details) on a complete blood count. Peripheral blood smear revealed ring forms of plasmodium falciparum and marked thrombocytopenia. Gross hepatosplenomegaly and scanty mesenteric lymphadenopathy were noted on abdominal ultrasonography. A clinical diagnosis of malaria with hyperreactive malarial splenomegaly (HMS) was made with a differential diagnosis of hematological malignancy. Subsequently, a bone marrow biopsy and aspirate were done. Smears were stained with Giemsa (see Figure 2A and B) while trephine biopsy was stained with H&E and these revealed Gaucher cells) which are weakly periodic acid Schiff (PAS) positive (see Figures 3A–D). Therefore, a diagnosis of Gaucher's disease was made although enzyme assay for glucocerebrosidase could not be done due to financial constraints.

She was managed for malaria with 3 doses of intravenous artesunate, dihydroartemisinin and piperaquine, paracetamol, and folic acid. Because of the lack of definitive treatment for Gaucher's disease, the family was counseled and

Figure 1 (**A**) Shows gross hepatomegaly and splenomegaly. (**B**) Shows the spleen after splenectomy was done.

Table 1 Shows Some of the Complete Blood Count Parameters and Other Blood Tests Done on Admission and How They Varied During the Follow-Up Period

Date	Parameters	Results	Normal Ranges
3/ February /2023 **(On admission)**	White blood cells	2.10 (10^3/μL)	3.10–15.00 (10^3/μL)
	Hemoglobin	5.9 (g/dl)	9.5–15.8 (g/dl)
	Mean corpuscle Volume	78.1 (fL)	68.0–98 (fL)
	Platelets	76 (10^3/μL)	126–438 (10^3/μL)
22/February/2023 (**After blood transfusion**)	White blood cells	2.52 (10^3/μL)	5.50–17.00 (10^3/μL)
	Hemoglobin	10.1 (g/dl)	9.5–13.5 (g/dl)
	Mean corpuscle volume	77.3 (fL)	76.0–92.0 (fL)
	Platelets	63 (10^3/μL)	150–400 (10^3/μL)
	Renal function		
	Creatinine	0.58 mg/dl	0.6–11 mg/dl
	Urea	20.0 mg/dl	10–50 mg/dl
	Liver enzymes		
	ASAT/GOT	23 U/L	0–37 U/L
	ALAT/GPT	20 U/L	0–42 U/L
	Alkaline phosphatase	152 U/L	64–306 U/L
	Peripheral blood	Malaria parasites (++ seen)	–
	Electrolytes		
	Sodium	137.8 mmol/L	135–145 mmol/L
	Potassium	5.01 mmol/L	3.5–5.5 mmol/L
	Chloride	107.2 mmol/L	98–108 mmol/L
07/July/2023 **(15 days post-splenectomy)**	White blood cells	14.4 (10^3/μL)	4.00–10.00 (10^3/μL)
	Hemoglobin	10.0 (g/dl)	11.0–15.0 (g/dl)
	MCV	74.5 (fL)	86.0–98.0 (fL)
	Platelets	666 (10^3/μL)	126–438 (10^3/μL)
31/July/2023 **(39 days post splenectomy)**	White blood cells	18.7 (10^3/μL)	4.00–10.00 (10^3/μL)
	Hemoglobin	11.4 (g/dl)	11.0–15.0 (g/dl)
	Mean corpuscle Volume	70.3 (fL)	86.0–98.0 (fL)
	Platelets	28 (10^3/μL)	126–438 (10^3/μL)

discharged on tranexamic acid per required need, fansidar two tablets monthly, and to be reviewed once every month. After 6 months of follow-up, splenectomy was done due to worsening hematological parameters. The procedure was successful, the spleen weighed 695g and was firm solid with no infarcts or discrete nodules seen (see Figure 1A and B).

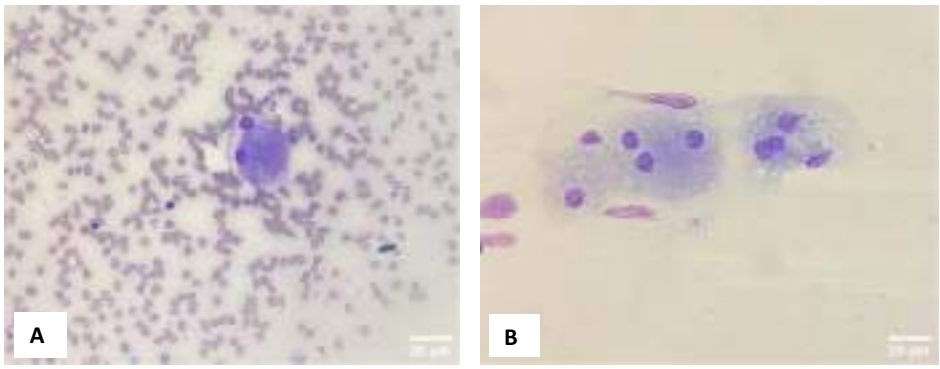

Figure 2 Both panels (**A** and **B**) are bone marrow smears showing large cells (macrophages) with multiple hyperchromatic eccentric and nuclei with abundant bluish cytoplasm (Gaucher cells) (X400, Giemsa stain).

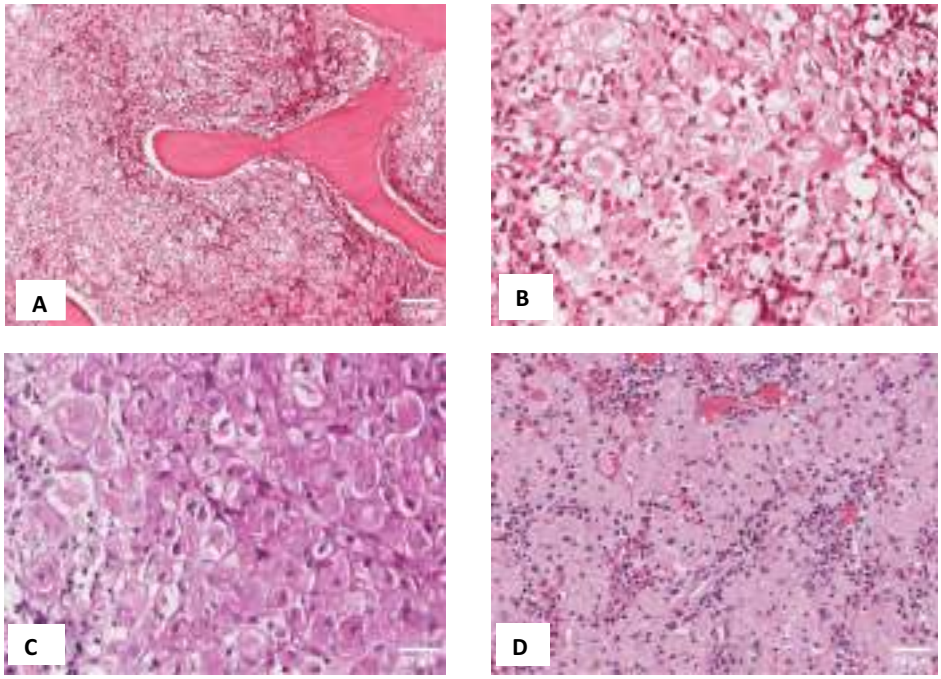

Figure 3 (**A**) Low power Images of the trephine biopsy showing a hypercellular marrow (100% cellularity) with numerous large cells with abundant pink cytoplasm crowding out all the hematopoietic cells. (**B**) high power view (x400) of the same marrow showing Gaucher cells with wrinkled tissue paper cytoplasm. (**C**) High power view of the trephine biopsy showing Gaucher cells weakly positive for periodic acid Schiff (PAS). (**D**) Histology section of the spleen showing sheets of macrophages (Gaucher cells) with abundant amphophilic cytoplasm and scattered residual lymphocytes.

Following splenectomy, the subsequent CBCs initially revealed an upsurge in the leukocytes (14,400/μL), platelets (666,000/μL), and near-normal hemoglobin (10.0g/dl) (done on day 2 weeks post-operatively). Five weeks postoperatively, she was still running a leukocytosis (18,700/μL), a normal hemoglobin (11.4g/dl), but had developed a thrombocytopenia (28,000/μL) (see Table 1). She also had developed severe bone pains in the pelvis and thighs with the inability to walk and stand. She also had episodic fevers. Lower limb x-ray revealed early skeletal deformities (Erlenmeyer flask deformity) (see Figure 4) involving the metaphysis of the femur. There was no evidence of osteonecrosis on x-ray. Her pain was managed by morphine in addition to physiotherapy. She still attends monthly reviews at pediatric oncology clinic and physiotherapy although the prognosis is still not known. Currently, she is back to school and doing fairly well. To further improve her management, we are now in contact with the International Gaucher Alliance who we hope will be key in the subsequent management of her condition. These events are summarized in the attached timeline figure (see Figure S1).

Discussion

GD type 1 is the most common form of the disease among the Jewish population with variable age of onset but is common before 20 years of age.[10,11] Patients have some residual activity of the enzyme glucocerebrosidase. It is characterized by the accumulation Gaucher cells in the bone marrow, spleen, liver, and kidneys. The central nervous system is however spared. Patients, therefore, present with bleeding diathesis, anemia, increased risk of infections, and abdominal distension due to organomegaly. In this case, there was worsening epistaxis, recurrent malaria infections, and abdominal distension. The previous recurrent attacks of malaria concealed the diagnosis of GD. The initial CBC revealed pancytopenia (see Table 1, first row). Because of the anemia, she had tachypnea, tachycardia, palpitations, and dizziness. These could also have been caused by the high fever of 38.4°C that she presented with because of malaria. She also had epistaxis at presentation which is attributed to thrombocytopenia. Thrombocytopenia can occur in severe malaria and this can easily confound the diagnosis.[12] However, in her case, she had been having these episodes of spontaneous epistaxis

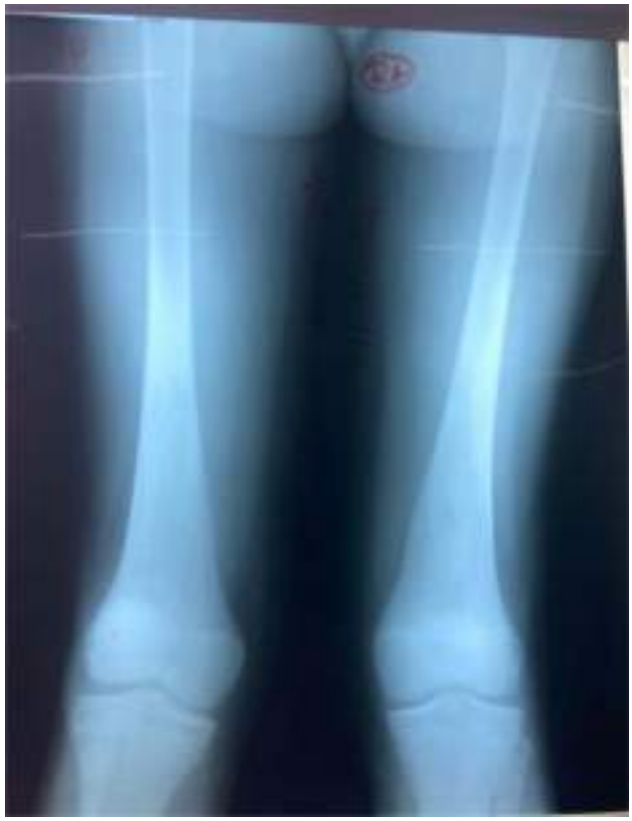

Figure 4 X-ray of the femur taken 5 weeks after splenectomy showing early musculoskeletal changes in the bones (Erlenmeyer flask deformity in the right femur).

for the last two years and had reportedly worsened with up to 4 episodes of epistaxis per day. Therefore, malaria infection could not solely explain the worsening epistaxis over the 2 years period.

As reported in all reported cases of type 1 GD, she also had characteristic hepatomegaly and splenomegaly of 12 cm and 8 cm (See Figure 1). Because we are in a malaria-endemic region, splenomegaly with a positive malaria test is always thought to be hyperreactive malarial splenomegaly (HMS). A recent systemic review revealed that up to 76% of splenomegaly in African countries is caused by HMS[13] as was also suspected in this case clinically. Because hepatosplenomegaly is not exclusive to HMS, other causes of hepatosplenomegaly need to be excluded to avoid misdiagnosis as it could easily have happened in this case.

Gaucher's disease can be clinically suspected by the presence of clinical signs and symptoms and the identification of Gaucher cells on histological examination of either splenic, liver, or bone marrow biopsy. These investigations are usually done to investigate other suspected conditions like hematological malignancies as was suspected in this case. In our case, a bone marrow aspirate was done for suspected leukemia. Giemsa stained smears revealed large cells with small eccentric nuclei with basophilic cytoplasmic inclusions which were suspected to be Gaucher cells. Examination of the H&E stained trephine biopsy showed abundant accumulation of macrophages crowding out all hematopoietic cells. The macrophages had eccentric condensed chromatin and abundant cytoplasm with wrinkled paper-like accumulations which were weakly PAS positive. Based on the presence of these cells and the clinical picture, a diagnosis of GD type 1 was reached at. However, although Gaucher cells are pathognomonic for the disease, pseudo-gaucher cells have been reported in several other conditions. Pseudo-gaucher cells are seen in chronic myeloid leukemia, acute myeloblastic leukemia, chronic lymphocytic leukemia, myeloma among others.[14]

Following detection of Gaucher cells, the diagnosis is confirmed by laboratory demonstration of deficiency of glucocerebrosidase enzyme activity in the white blood cells (Beta-glucosidase leukocyte blood test). Patients with type 1 GD usually have some residual activity of the enzyme. Also, the test may not be helpful in carriers for which genetic

testing is required to detect variants in the GBA1 gene.[1,5] In the case presented, enzyme testing was not done and it is not available in the country. Attempts were made to have it done but the only available option was shipping the sample to India at a cost that the family could not afford.

Enzyme replacement therapy (ERT) is the mainstay treatment strategy, especially for type 1 GD. Recombinant glucocerebrosidase enzymes (imiglucerase, velaglucerase alfa, and taliglucerase alfa) are currently in use; however, these are not available in most underdeveloped countries. In our setting, treatment is largely supportive as indicated in the case above and in several cases reported in Kenya.[6] After 6 months of supportive care, cytopenia and organomegaly were worsening and therefore a total splenectomy was done despite the adverse consequences. Splenectomy is indicated as one of the treatment options in patients not receiving ERT but the prognosis after the procedure is still debatable. There may be an improvement in hematological abnormalities,[15] however, the disease tends to worsen in other organs with an increased risk of pulmonary hypertension and malignancy[16] as seen in the case above. After splenectomy, the hematological parameters showed an improvement with hemoglobin in the normal range. The white blood cells improved beyond the normal upper limit and this coincided with a fever although there were no other features of possible infection. Contrary to hemoglobin and white blood cells, platelets remained consistently low, lower than the pre-splenectomy values. Additionally, she started experiencing bone pains with evidence of Erlenmeyer flask deformity but without osteonecrosis. The pain could be a result of sudden increase in the Gaucher cells in the bones following splenectomy. Ultimately, we predict that she will suffer pathological fractures. Other recommended treatment options include substrate reduction using either eliglustat or miglustat. These block the production of glucocerebrosides thus preventing their accumulation in the macrophages.[5]

Limitations

Confirmation of GD requires enzyme assay to establish deficiency of glucocerebrosidase. In our setting, the test could not be done. The available option was provided by a private laboratory that suggested to ship the blood sample to India, at a cost that could not be afforded by the family.

Conclusion

GD is a rare disease among the Africans. Its symptoms can easily be mistaken for other conditions like HMS seen is malaria-endemic areas. Diagnosis and access to standard care for rare conditions like GD is still a challenge in developing countries like Uganda. Although ERT is the standard of care for GD, splenectomy is still an option especially in low resource settings where ERT is unavailable. Although splenectomy may result in an improvement of the hematological parameters, it should only be done if it is indispensable because of the deterioration that may follow.

Data Sharing Statement

The data and materials of this case report are available from the corresponding author upon request after approval from the Pathology Department and Mbarara Regional Referral Hospital.

Consent and Ethical Approval

Parental consent was obtained to authorize us to publish the case details and other images obtained from her daughter as part of the case. The mother also gave consent to use the remaining specimens for any further research purposes in the future. The head of the pathology department also provided us with clearance to use the laboratory and the specimen. Institutional approval was not required.

Patient Perspective

This being a rare genetic condition, the family was really worried and concerned about their daughter's condition. What was even more disturbing was the fact that it had no definitive cure and the recommended drugs were not available in the country. The family was counseled and educated about the pattern of inheritance and the type of care that can be provided to them. Fortunately, they are very adherent to treatment and have followed the follow-up schedule to the dot and so far they are satisfied with the care given despite the dismal outcome that the condition bears.

Acknowledgment

We acknowledge the patient and her family for allowing us to take pictures of their valuable body parts and to use their specimen publication for future research purposes. We also acknowledge the pathology department of Mbarara University and the Oncology Clinic at Mbarara regional referral hospital for the caring for sick.

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Disclosure

The author(s) report no conflicts of interest in this work.

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Assessment of Erythrocyte Osmotic Fragility and Its Determinants, and Comparison of Hematological Indices Among Type 2 Diabetes Mellitus Patients on Follow-Up at Jimma Medical Center, Southwest Ethiopia

Zenebe Tujara, Wondu Reta, Eyasu Tadesse, Ifa Dereje & Mekonnen Tesfa

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ORIGINAL RESEARCH

Assessment of Erythrocyte Osmotic Fragility and Its Determinants, and Comparison of Hematological Indices Among Type 2 Diabetes Mellitus Patients on Follow-Up at Jimma Medical Center, Southwest Ethiopia

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Background: Diabetes Mellitus (DM) is one of the most prevalent non-communicable diseases worldwide. Approximately 9.3% of the general population was estimated to have DM globally in 2019. Erythrocyte osmotic fragility (EOF) in hyperglycemic patients is expected to increase and determine the rate of erythrocyte hemolysis.

Purpose: This study aimed to assess erythrocyte osmotic fragility (EOF) and its determinants and to compare hematological indices among T2DM patients on follow-up at the Jimma Medical Center (JMC), Jimma, Southwest Ethiopia.

Methods: A facility-based cross-sectional study involving 124 participants (each 62) of T2DM patients and controls was conducted from October to November 2020 using a structured questionnaire. 5 mL of venous blood was drawn to assess OF, complete blood count, and blood glucose levels. EOF was investigated using a series hypotonic solution of NaCl. The supernatant of the centrifuged sample was transferred to cuvette test tubes, and the hemolysis stage was read on a spectrophotometer. The collected data were coded and entered into Epi-data Version 3.1. The analysis was performed using SPSS Version 23.

Results: Compared with non-diabetic controls, patients with T2DM had significantly increased EOF. FBG >126mg/dl (AOR=7.741, 95% CI: 1.562–38.360), PPBG >200 mg/dl (AOR=7.576, 95% CI: 1.519–37.791), RDW (AOR=4.558, 95% CI: 1.136–18.284) were significantly associated with abnormal EOF. A statistically significant increase in total white blood cells and absolute neutrophil counts ($P < 0.001$) were observed in T2DM patients. From RBC indices, red blood cell distribution width (RDW) and mean corpuscular volume (MCV) were significantly increased in T2DM patients ($P < 0.001$).

Conclusion: This study suggests that EOF was greater in patients with T2DM than in non-diabetic controls and was determined by FBG, PPBG, and RDW. The study also demonstrated that hematological index alterations were higher in T2DM subjects than in non-diabetic controls.

Keywords: diabetes, osmotic fragility, hematological indices, RBC

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders with various etiologies characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent noncommunicable diseases worldwide.[1] The prevalence of DM is steadily increasing worldwide and can be attributed to recently released updates from the World Health Organization (WHO), which states that the number of people with DM has quadrupled to an estimated 422 million adults worldwide since the publication of the first report by the WHO in 1980.[2]

In 2017, it was estimated that 451 million people (aged 18–99 years) had DM worldwide. This number is expected to increase to 693 million by 2045. In 2017, approximately 5 million deaths worldwide were attributable to DM in the age range

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of 20–99 years.[3] According to the World Health Organization (WHO), DM is becoming a global pandemic.[4] DM encompasses a heterogeneous group of disorders characterized by hyperglycemia associated with multiple disorders, including metabolic, cellular, and blood disturbances, leading to vascular complications.[5] T2DM, which is previously referred to as "noninsulin-dependent DM" or "adult-onset DM", accounts for ~90–95% of all DM. It encompasses individuals with insulin resistance and is usually a relative (rather than absolute) insulin deficiency.[6] T2DM associated with a poor lifestyle is a primary factor leading to a progressive reduction in physical activity and changes in dietary habits. Therefore, a greater percentage of the population will be overweight or obese. It is one of the most common chronic diseases worldwide, affecting approximately 7% of the general population, is a major public health challenge in the 21st century.[3,7] The chronic hyperglycemia in DM, especially when poorly controlled, causes long-term damage, dysfunction, and failure of different organs of the body like the eyes, kidneys, nerves, blood, and blood vessels.[8]

Patients with T2DM have a significantly higher erythrocyte osmotic fragility (EOF) than matched non-diabetic subjects, and fasting plasma glucose is the strongest correlate of increased EOF in the patient group.[9,10] EOF was greater in T2DM, which is positively correlated with glycosylated hemoglobin. EOF determines the rate of hemolysis in erythrocytes.[9,11] Several studies have shown that abnormal erythrocyte membrane formation in hyperglycemia may be associated with increased RBC fragility, leading to anemia.[12] The pathogenesis of diabetic complications is complex; however, hyperglycemia appears to be the primary mechanism of disease progression. Chronic exposure to glucose affects human cells. However, erythrocytes have gained special interest from the research community owing to their long lifespan (120 days).[13] Abnormal glycation, which can adversely affect hemoglobin and membrane proteins in erythrocytes, has been shown to correlate with reduced membrane fluidity. High levels of glycosylated hemoglobin have been found to correlate with decreased deformability of erythrocytes.[14] Researchers have observed that EOF is increased in chronic diabetic patients in the Indian population. They suggested that EOF is related to the duration of DM.[15]

Anemia is a highly prevalent condition in people with T2DM. It is defined by the WHO as hemoglobin concentration below the threshold for women (>15 years) <12.0 g/dl and men (>15 years) <13 g/dl. Generally, anemia in chronic diseases such as DM is normocytic normochromatic, although in a few cases, microcytosis and hypochromia also occur.[16]

According to the WHO definition, up to nearly 30% of patients with T2DM and nearly one in four (23%) patients with type 1 or T2DM have anemia, especially those with reduced kidney function.[17] One of the proposed mechanisms for anemia in T2DM is the formation of abnormal erythrocyte cell membranes, which lead to reduced surface area-to-volume. A reduced surface-area-to-volume ratio might result in decreased deformability and thereby influence the splenic sequestration of erythrocytes, leading to increased destruction and anemia.[9] T2DM represents a major public health challenge because of its continuously increasing prevalence. According to the International Federation of Diabetes (IDF), there was an increased by approximately 64% in the worldwide prevalence. It is estimated that approximately 151 million to 415 million patients were affected by T2DM from 2000 to 2015, respectively, and it is expected to surge from this point by 36% until 2040, reaching approximately 642 million.[18] Additional data also show that worldwide, there is a projected increase in the prevalence of DM from 382 million (8.3%) in 2013 to 592 million (10.1%) in 2035. The prevalence of this phenomenon in developing countries is increasing, and its number is even more striking. This is especially true in areas where populations quickly embrace western lifestyles.[19] The future looks concerning for developing countries, as they are projected to bear a staggering 77% of the global burden of the DM epidemic in the 21st century. This unfortunate reality can be attributed to factors such as population growth, unhealthy diet, obesity, and sedentary lifestyle.[20] Moreover, in 2017, the projected national DM (20–79) prevalence in Ethiopia, estimated the IDF Atlas, was 5.2%.[21] Although several studies in different countries have suggested an increase in EOF in T2DM patients, few studies have been conducted in the Ethiopian population. Therefore, the aim of this study was to assess EOF and its determinants and to compare hematological indices among T2DM patients on follow-up at the Jimma Medical Center (JMC), Jimma, Southwest Ethiopia.

Materials and Methods

Study Area and Study Period

The study was conducted at the diabetic clinic of the Jimma Medical Center (JMC). Geographically, it is located in Jimma City, 352 km southwest of Addis Ababa, the capital of Ethiopia. JMC is the only teaching hospital in southwest Ethiopia that

provides services for approximately 15,000 inpatients, 160,000 outpatient attendants, 11,000 emergency cases, and 4500 deliveries annually, with a catchment population of approximately 15 million people. The study was conducted between September and November 2020.

Study Design and Populations

A hospital-based comparative cross-sectional study was conducted from September to November 2020, and the source population included all T2DM patients on follow-up at the JMC 2020. All T2DM patients on follow-up at the JMC, with age- and sex-matched healthy controls, were included as the study population. The inclusion criteria were as follows: all patients with T2DM aged greater than 18 years and who had follow-up for at least 3 years at the diabetic clinic were included in the study. Patients with known chronic renal disease, pregnant mothers, and those who were appointed for a second time during the study period were excluded from the study.

Sample Size Determination and Sampling Techniques

The study participants were assumed to be homogeneous. No significant differences were observed among the study participants. Considering these assumptions, a consecutive sampling technique was employed to select both groups, until the desired number of participants was achieved.

The double population mean formula was used to calculate the sample size using Open Epi version 2, open-source calculator by considering the following assumptions: 95% confidence interval (two-sided), 80% power, and the ratio of cases to control group was 1:1. The mean and standard deviation (SD) of the hematocrit (%) ratio for the T2DM and control groups in a study conducted in Italy[9] were 39.7 and 5.5 for the T2DM group and 42 and 3.3 for the control group, respectively. The sample size was determined to be 62 for each group.

Data Collection Methods

Structured questionnaires were used to collect baseline data. Trained clinical nurses who provided healthcare at a chronic outpatient clinic collected blood samples. After obtaining consent from the participants, approximately 5 mL of venous blood was collected into EDTA tubes at the time of the clinic visit (follow-up). When taking blood, health workers wore well-fitting, non-sterile gloves, and hand hygiene was performed before and after each procedure. The EOF status was measured using spectrophotometry. To perform the fragility test of RBC, a series of a hypotonic solutions of NaCl were prepared with different strengths in 14 test tubes, numbered serially (0.0, 0.10, 0.20, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.75, 0.85, and 0.9%), and kept in the test tube rack. One drop of anticoagulant-mixed blood was added to each test tube and mixed gently by inverting the test tubes. After waiting for about half an hour of hemolysis, the test tubes were centrifuged for approximately 5 min at 2500 rpm using Thermo Scientific (MR-23i, Germany). After centrifugation, the supernatant was transferred to a cuvette and the hemolysis stage was measured using a spectrophotometer and recorded in a datasheet. In addition to routine laboratory investigations, experienced laboratory professionals performed a complete blood count (CBC) using a Sysmex XT-1800i (Sysmex, Japan) and blood glucose status (FBG and PPBG). Other demographic and DM-related data, such as current age, sex, residence, diabetes duration, and age at onset of DM, will be gathered through face-to-face interviews in a separate room for privacy.

Data Processing and Analysis

The collected data were entered into Epi data version 3.1 and exported to SPSS version 23 for analysis. Crosschecking and cleaning were performed to avoid missing values, outliers, and inconsistencies before the analysis. A descriptive analysis was performed to determine the frequency of sociodemographic and other variables. The mean ± SD of all study groups was compared for statistical significance using an independent *t*-test. Pearson's correlation test was used to identify the correlation between continuous variables, and logistic regression was used to compute the relationship between independent and dependent variables. The results of continuous data are presented as means and standard deviations. Statistical significance was set at $P < 0.05$.

Data Quality Control

Before data collection, the questionnaire was translated into Afan Oromo and Amharic, and back-translated into English to maintain consistency. Data collectors were trained, adequately oriented, and data collectors were supervised by the principal investigator. Data were collected using a questionnaire, and the information provided by the patients was crosschecked with the recorded data for consistency and completeness. The validity, accuracy, and precision of these tools were evaluated. To maintain the quality of the laboratory tests, standard operating procedures were followed for the collection of blood specimens for research. CBC analysis was performed according to the hospital protocol. After collection of the blood samples, labeling was performed on the sample and request paper with the same identification number. Data quality was maintained by daily on-site supervision during the data collection period.

Ethical Considerations

Ethical clearance for the study was obtained from the Jimma University Institute of Health Institutional Review Board. Written letter of permission for data collection was provided to the Jimma University Medical Center administrative office, endocrinology department, and the hospital's laboratory unit. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. Written informed consent was obtained from all the participants. Throughout this research, investigators, data collectors, and advisors carefully applied all preventive measures against COVID-19. Physical distance was intellectually maintained at any time during face-to-face contact. Sanitizers, gloves, and facemasks were made available. Laboratory equipment was sanitized before and after the procedure. All participant information was kept confidential via a coding system and no direct benefit was given to the participants. Experienced laboratory technicians performed venous blood collection as a routine, acceptable clinical practice, with no potential risk. Blood samples were collected from a dedicated location to ensure patient comfort and privacy. The laboratory test results and their implications were communicated to the patients and their care providers immediately during the next follow-up to assist them in improving diabetic care.

Results

Characteristics of Study Participants

A total of 124 participants (62 T2DM patients and 62 controls) were included in this study. Of the total T2DM patients, 36 (58.1%) were males and 26 (41.9%) were females. Similarly, among 124 healthy controls, 36 (58.1%) and 26 (41.9%) were males and females, respectively. The mean age (mean ± SD) was 50.71±12.8 and 48.29±8.1 years for T2DM patients and controls. The majority of the T2DM participants were farmers (32.3%) from rural (51.6%), and their age was >49 (54.8%) (Table 1).

Comparison of Various Parameters of DM & EOF Among Study Participants

Mean (±SD) FBS was significantly higher (P<0.001) in T2DM (156.92 ±39.60) g/dl than the control group (93.34 ± 8.079). Mean (±SD) of PPBS was significantly higher (P<0.001) in T2DM (197.03 ± 9.46) g/dl than in the control group (116.77 ± 5.894). Regarding EOF, mean values of NaCl solution for initial, medial, and complete hemolysis were 0.540 ±0.055%, 0.455 ± 0.043%, and 0.382 ± 0.035% and 0.456 ± 0.023%, 0.40 ± 0.014%, and 0.345 ± 0.014% between cases and controls respectively (Figure 1). A significant difference (P<0.001) was found between the groups for all types of hemolysis (Table 2). Among study participants, 80.65% and 6.5% of T2DM and controls had abnormal hemolysis, respectively (Table 3).

Comparison of Hematological Profile Among Study Participants

Regarding the white blood cell white blood cell (WBC) indices, a statistically significant increase in total WBC counts ($P < 0.001$) and absolute neutrophil count ($P < 0.001$) was observed in T2DM patients compared to the control group. Among the RBC indices, red blood cell distribution width (RDW) and mean corpuscular volume (MCV) showed statistically significant increases in T2DM ($P < 0.001$) patients. In addition, statistically significant increases in platelet counts ($P < 0.001$) were observed in T2DM patients compared with those in the control group (Table 4).

Table 1 Socio-Demographic Characteristics and Substance Use Behavior of the Study Participants at JMC, Southwest Ethiopia, 2020 (n=124)

Variables		T2DM (n=62)		Controls (n=62)	
		Frequency	Percentage (%)	Frequency	Percentage (%)
Age category	19–28	2	3.2	2	3.2
	29–38	10	16.1	4	6.5
	39–48	16	25.8	12	19.4
	>49	34	54.8	44	71.0
Gender	Male	36	58.1	36	58.1
	Female	26	41.9	26	41.9
Educational status	Illiterate (unable to read and write)	15	24.2	8	12.9
	Primary (1–8)	28	45.2	18	29.0
	Secondary (9–10)	13	21.0	24	38.7
	Higher education	6	9.7	12	19.4
Occupation	Farmer	20	32.3	11	17.7
	House wife	16	25.8	14	22.6
	Government employee	6	9.7	15	24.2
	Private employee	9	14.5	18	29.0
	Unemployed	1	1.6	4	6.5
	Student	2	3.2	11	17.7
	Others	8	12.9	14	22.6
Residence	Urban	30	48.4	41	66.1
	Rural	32	51.6	17	27.4
Chronic complication	Yes	27	43.5	0	0
	No	35	56.5	62	100
Alcohol drinking habit	Yes	6	9.7	9	14.5
	No	56	90.3	53	85.5
Smoking habit	Yes	2	3.2	8	12.9
	No	60	96.8	54	87.1

Correlations of EOF Status with FBG and PPBG Among Study Participants

The correlation of FBS and PPBS with initial, medial, and complete hemolysis was significantly positive in the T2DM group (Table 5). Pearson's correlation also indicated that the Hb level was significantly ($P<0.001$) negatively correlated with initial hemolysis (Figure 2).

Correlations of EOF Status with Hematological Parameters Among T2DM Patients

On Pearson correlation, some hematological parameters, such as RDW (fl), MCHC (g/dl), and Platelet (103/μL) in T2DM patients showed a strong positive correlation with abnormal hemolysis (Table 6).

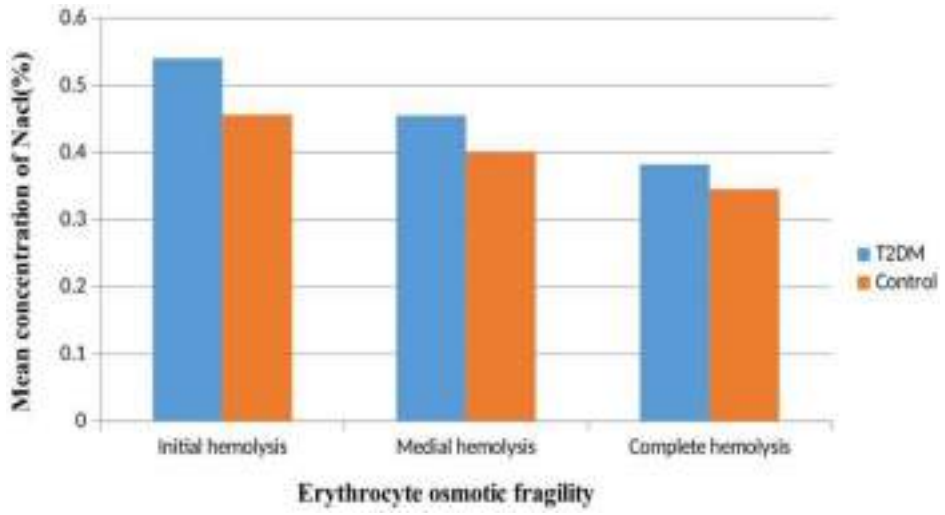

Figure 1 The mean concentration of NaCl of OFT in Diabetic and non-diabetic study participants at JMC, Southwest Ethiopia, 2020 (n=124).

Factors Determine Erythrocyte Osmotic Fragility

To evaluate the association of each independent variable with the occurrence of EOF, binary logistic regression was performed between the occurrence of EOF (dependent variable) and selected factors (independent variable). To identify the most significant determinants of EOF, factors that showed a P-value ≤0.25 in the bivariate analysis, were candidates for the multivariate logistic regression model. Analyses revealed a statistically significant association between FBG (mg/dl), PPBG (mg/dl), RDW, and abnormal EOF. Accordingly, patients with FBG >126 mg/dl were approximately eight times more likely to have abnormal EOF than those with FBG <126mg/dl (AOR=7.741, 95% CI: 1.562–38.360). Similarly, those who have PPBG >200 mg/dl were approximately eight times more likely to have abnormal EOF than those with <200 mg/dl (AOR=7.576, 95% CI: 1.519–37.791). Additionally, patients who have RDW >40 FL were approximately five times more likely to have abnormal EOF than those with RDW <40 FL (AOR=4.558, 95% CI: 1.136–18.284) (Table 7).

Table 2 Comparison of Various Parameters of Diabetes & Erythrocyte Fragility Among Test Group and Control Group of the Study Participants at JMC, Southwest Ethiopia, 2020 (n=124)

Variables	T2DM		Controls		P values*
	(Mean ± SD)	Range	(Mean ± SD)	Range	
FBG (mg/dl)	156.92 ±39.60	135	93.34 ± 8.079	45	< 0.001
PPBG (mg/dl)	197.03 ± 9.46	120	116.77 ± 5.894	35	<0.001
Initial hemolysis (% of NaCl)	0.540 ± 0.055	0.20	0.456 ± 0.023	0.10	< 0.001
Median hemolysis (% of NaCl)	0.455 ± 0.043	0.20	0.40 ± 0.014	0.050	< 0.001
Complete hemolysis (% of NaCl)	0.382 ± 0.035	0.15	0.345 ± 0.014	0.050	< 0.001

Note: *P values: Indicate significance of the test.

Table 3 Nature of Hemolysis in the Study Groups at JMC, Southwest Ethiopia, 2020 (n=124)

Hemolysis	T2DM n = 62		Controls n= 62	
	No	%	No	%
Normal hemolysis	12	19.35	58	93.5
Abnormal hemolysis	50	80.65	4	6.5

Table 4 Comparison of Hematological Indices of the Study Participants at JMC, Southwest Ethiopia, 2020 (n=124)

Variables	Mean ± SD (T2DM)	Mean ± SD (Controls)	P-value
White blood cell indices			
WBcs (10^3 /μL)	6.60 ± 1.74	5.47± 0.63	< 0.001
Lymphocytes (10^3/μL)	2.36 ± 3.17	2.01±0.20	0.388
Neutrophils (10^3/μL)	3.76 ±1.59	2.50 ± 0.90	< 0.001
Monocyte (10^3/ μL)	0.53 ± 0.17	0.51 ± 0.10	0.357
Eosinophil (10^3/ μL)	0.28 ± 0.49	0.21±0.17	0.337
Basophil (10^3/ μL)	0.02 ± 0.01	0.03 ± 0.02	0.084
Red blood cell indices			
RBCs (10^6/ μL)	4.92 ±0.54	5.85± 6.09	0.231
Hgb (g/dl)	14.20 ± 1.70	14.70 ± 1.10	0.029
Hct (%)	42.80 ± 4.13	41.70 ± 1.77	0.052
MCV (fl)	89.40 ± 2.450	86.75 ± 4.12	< 0.001
MCH (pg)	29.96 ± 1.480	30.57 ± 1.20	0.014
MCHC (g/dl)	34.52 ± 1.19	35.40 ± 7.10	0.350
RDW (Fl)	44.30 ± 4.01	38.30 ± 1.34	< 0.001
Platelet	279.03± 56.20	235.18± 33.70	< 0.001

Note: P-value <0.05 is considered statistically significant.

Table 5 Pearson's Correlations (r) of EOF Status with FBG and PPBG Among T2DM Patients and Healthy Controls at JMC, Southwest Ethiopia, 2020 (n=124)

Variables	T2DM Group		Control Group	
	FBG r(P)	PPBG r(P)	FBG r(P)	PPBG r(P)
Initial hemolysis	0.316*(0.012)	0.907**(0.000)	− 0.002(0.986)	−0.073(0.571)
Medial hemolysis	0.338**(0.008)	0.477**(0.000)	0.165(0.199)	0.105(0.419)
Complete hemolysis	0.308*(0.015)	0.442**(0.000)	0.122(0.346)	0.258*(0.043)

Notes: **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Discussion

This study was conducted to assess EOF and its determinants and to compare hematological indices among patients with T2DM. The findings of this study revealed significant differences in EOF between the T2DM and control groups. This study indicated 80.65% and 6.5% of T2DM and controls showed abnormal hemolysis, respectively.

The mean values of NaCl solution for initial, medial, and complete hemolysis in T2DM were 0.540 ±0.055, 0.455 ± 0.043, and 0.382 ± 0.035, respectively. In control group, it was 0.456 ± 0.023, 0.40 ± 0.014 and 0.345 ± 0.014. A significant difference (P<0.001) was observed between the two groups for all types of hemolysis. This study is in line with studies conducted in Nigeria and India, which confirmed that EOF increased in patients with diabetes.[10,15,22]

The results of this study indicate that EOF is negatively correlated with hemoglobin levels (Figure 2), which is consistent with the results of studies conducted in Southern India and Egypt.[11,23] This finding implies that patients with T2DM are at risk of developing hemolytic anemia. A moderate-to-strong positive correlation was observed between all forms of EOF and blood glucose (FBG and PPBG) (Table 5), which was simulated in a study conducted in Italy.[9] This finding emphasizes the importance of strong glycemic control in minimizing EOF and consequent problems in DMs.

Research evidence suggests that hematological indices are altered in patients with T2DM.[24] In patients with DM, persistent hyperglycemia exposes RBCs to elevated glucose concentrations, resulting in glycation of hemoglobin, prothrombin, fibrinogen, and other proteins involved in clotting mechanisms.[25] In this study, there was a significant

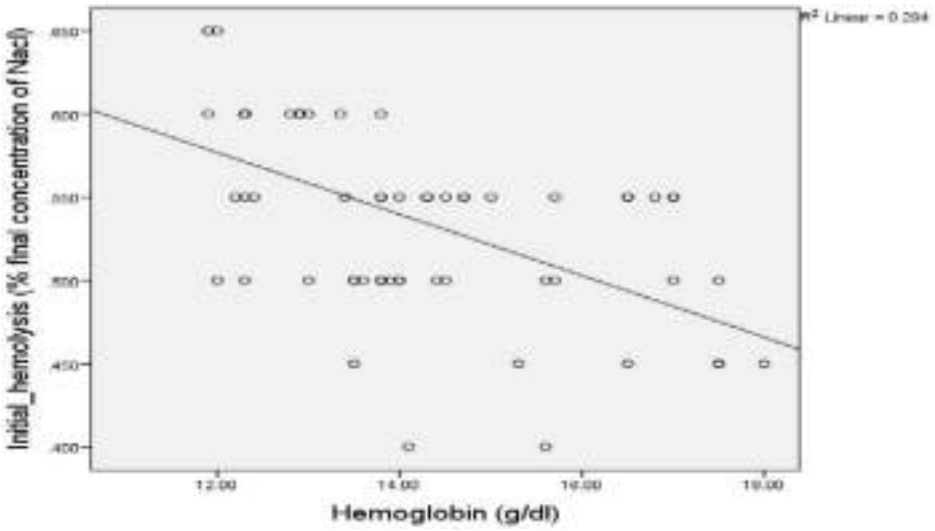

Figure 2 Correlation between Hb (g/dl) and initial hemolysis per percentage of NaCl concentration in samples of type 2 diabetic patients at JMC, Southwest Ethiopia, 2020 (r= - 0.542 P <0.001).

reduction in hemoglobin levels compared to the control group (P<0.05). This finding is in line with those of studies conducted in the UK and Bangladesh, which indicated that patients with DM were anemic.[26–28] Our study also revealed that, among other RBC indices, RDW and MCV values were significantly different between the T2DM and control groups (Table 4).[29,30] A high RDW indicates impairment of erythropoiesis, reflecting chronic inflammation and increased levels of oxidative stress, both of which are significant signs of T2DM that result in variations in RBC size.[31] This study also included a comparison of platelet counts between control and diabetic patients. The study showed a significant difference in platelet counts between the diabetic and control groups (p<0.001). Our findings are in line with those of a study conducted at Lagos State University Teaching Hospital, Nigeria, which revealed a higher mean platelet count in patients with diabetics than in non-diabetic controls.[32]

Insulin resistance appears to increase platelet activation, which is consistent with the increased platelet reactivity.[33] However, in contrast to our findings, a study conducted at the University of Gondar indicated that despite the increase in platelet count in T2DM, the duration it was not significant.[34] Duration of DM and other environmental factors may account for the variability of platelet count across studies. In the current study, it was observed that there was a significant increase in both white blood cell (WBC) and neutrophil counts in the T2DM group compared to the control group. A study conducted in Southern Taiwan also indicated that the WBC count is elevated in T2DM patients and may contribute to vascular complications.[35] High WBC count may be associated with a decline in insulin sensitivity. These data indicate that inflammation plays a role in insulin resistance and the subsequent development of T2DM.[36] Our study also suggested an increase in the absolute monocyte and eosinophil counts; however, no significant difference was found between the two groups. This finding is in agreement with previously published reports on the risk of atherosclerosis in a community and study conducted at the University of Gondar.[34,37]

Biological evidence suggests that inflammation may induce T2DM, and epidemiological studies have shown an association between a higher WBC count and T2DM.[29,38] Surprisingly, RDW, MCHC, and Platelet count showed

Table 6 Pearson's Correlations (r) of EOF Status with Hematological Parameters Among T2DM Patients at JMC, Southwest Ethiopia, 2020

Variables	RDW(fl) r(p)	MCHC (g/dl) r (p)	Platelet (10^3/μL) r(p)
Abnormal hemolysis	0.895**(0.000)	0.897**(0.000)	0.571**(0.000)

Note: ** Correlation is significant at the 0.01 level (2-tailed).

Table 7 Multi-Variable Logistic Regression of Variables Associated with EOF Among T2DM Patients at JMC, Southwest Ethiopia, 2020 (n=124)

Variables	Erythrocyte Osmotic Fragility		COR (95% CI)	P-value	AOR (95% CI)	P-value
	YES	NO				
Duration of disease						
< 5 years	6	5	1*		1*	
> 5 years	44	7	**5.238** (1.254–21.886)	0.023	**4.729**(0.791–28.280)	0.089
FBG (mg/dl)						
<126	10	8	1*		1*	
>126	40	4	**8** (2.001–31.988)	0.003	**7.741**(1.562–38.360)	0.012**
PPBG (mg/dl)						
<200	11	8	1*		1*	
>200	39	4	**7.091** (1.794–28.021)	0.005	**7.576**(1.519–37.791)	0.014**
RDW (FL)						
< 40	10	7	1*		1*	
> 40	40	5	**5.60** (1.465–21.400)	0.012	**4.558**(1.136–18.284)	0.032**
Chronic compaction						
Yes	23	9	1*		1*	
No	27	3	**3.522** (0.851–14.571)	0.082	**3.176**(0.712–14.165)	0.130

Notes: 1*References: Category; **Shows P<0.05, =significant association with multivariable logistic regression. Bolded font: Crude and adjusted odd ratio.
Abbreviation: FL, femtoliter.

a strong positive correlation with abnormal hemolysis in the current study (Table 6). This finding suggests that red blood indices and platelet count alterations are one of the causes of EOF due to viscoelasticity, and that the shape of red blood cells is disturbed in T2DM.[39]

Our study also attempted to identify the factors that determine EOF. This study indicated that the odds of developing abnormal EOF among T2DM patients with FBG levels ≥126 mg/dl were eight times more likely than among those with FBG levels <126 mg/dl. This study also showed greater odds for the occurrence of abnormal EOF among T2DM patients with PPBG ≥ 200 mg/dl than among those with PPBG < 200 mg/dl. This finding is in line with those of studies conducted in Italy, Nigeria, and Sudan.[9,10,40] This may be because the chronic increase in blood glucose levels causes oxidative modifications of membrane-bound proteins in RBCs, which affects the strength and functional properties of the membrane. Thus, the RBCs membrane weakens and cannot withstand mild hypotonic conditions, leading to increased EOF. In addition, our study suggested that patients with RDW > 40 FL were approximately five times more prone to develop abnormal EOF than those with RDW < 40fl.

Conclusion

The results of this study indicate that EOF is greater in T2DM participants than in non-diabetic controls and was determined by FBG, PPBG, and RDW. This study also demonstrated that hematological index alterations were higher in T2DM subjects than in non-diabetic controls and indicated a strong positive correlation between RDW, MCHC, platelets, and EOF.

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Disclosure

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

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ORIGINAL RESEARCH

Hematological Parameters of Gasoline Station Workers at Hosanna Town, Southwest Ethiopia: A Comparative Cross-Sectional Study

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Background: Human exposure to benzene is associated with many adverse health effects. It is mainly related to impairment of the hematopoietic system and bone marrow suppression, causing abnormalities in hematological parameters. However, the reports obtained from different studies are contradictory, and there are little data regarding the hematological parameters of gas station workers in the study area. Therefore, this study aimed to evaluate the hematological parameters of gas station workers in Hosanna town, southwest Ethiopia, from May 01 to June 15, 2020.

Methods: A comparative cross-sectional study was conducted by involving 180 (60 gas-stations workers and 120 controls) participants. Socio-demographic and related data of the study participants were collected using a pre-tested structured questionnaire through face-to-face-interviews. All phases of quality assurance were maintained, and hematological parameters were determined using Uni-Cel DxH 800 automated hematological analyzer. Independent sample *T*-test, Mann–Whitney *U*-test, and one-way ANOVA were used for data analysis. Statistical significance was declared at P<0.05.

Results: Statistically significant difference was observed in hematological parameters of gasoline-workers and control groups. The mean of red blood cell count among gasoline-workers was significantly reduced as compared to control groups (p=0.007). In addition, the median of hemoglobin levels among gasoline-workers was significantly decreased as compared to the control groups (p=0.001). In contrast, a significant increase was observed in median of absolute eosinophil count among the gasoline-workers as compared to control groups (p=0.01). The mean of mean cell volume was significantly decreased with respect to the duration of work experience (p=0.04).

Conclusion: In this study, a statistically significant difference was observed in some hematological parameters of gas station workers compared to the control group. Therefore, medical observation and periodic medical check-ups of the hematological profile should be considered to prevent the development of medical complications.

Keywords: benzene, Ethiopia, gasoline workers, hematological parameters, Hossana

Introduction

Hematopoiesis refers to the commitment and differentiation processes that lead to the formation of blood cells from multipotent hematopoietic stem cells (HSCs) in the bone marrow. Extramedullary hematopoiesis can also occur in other tissues such as the liver and spleen. The ability to differentiate into all hematopoietic lineages and maintain their self-renewal capacity is a key characteristic of stem cells.[1,2] Various factors can affect the formation of blood cells in target tissues. These factors encompass metabolic abnormalities, infections, inflammation, as well as exposure to gasoline.[3–5] Under stressful conditions, such as exposure to gasoline, hematological parameters are likely to undergo rapid and easily detectable variations, making them valuable for assessing one's health status.[6]

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Human exposure to benzene has been associated with a variety of adverse health effects, primarily related to impairment of the hematopoietic system with bone marrow failure, including pancytopenia and aplastic anemia, increased risk of developing acute myeloid leukemia and morphological changes in blood cells.[7] Benzene is an aromatic hydrocarbon and a natural component of crude oil and natural gas. It is a colorless, highly flammable liquid with a sweet odor. Systemic exposure to benzene may cause acute and chronic clinical disorders associated with a risk of hematologic abnormalities, including leukemia, lymphoma, and chromosomal aberrations.[8] Several mechanisms are involved during benzene-induced toxicity including oxidative stress, DNA damage, cell cycle disturbance, and apoptosis.[9]

The production of benzene metabolites takes place mainly in the liver, where they are then transported to the bone marrow. Benzene is metabolized to phenol, its main metabolite, by the liver enzyme, cytochrome P4502E1 (CYP2E1), via benzene oxide. It is then metabolized by CYP2E1 to hydroquinone (HQ). Hydroquinone is transported to the bone marrow and oxidized to a number of metabolites that can accumulate in the bone marrow, which are then bioactivated by myeloperoxidase and other heme protein peroxidases to form semiquinone, quinine, and benzochinone. This causes formation of reactive oxygen species (ROS). These reactive oxygen species can affect signaling cascades by altering the activity of certain protein kinases and transcription factors.[10] Finally, adverse effect of benzene is associated with bone marrow failure and hematological malignancies.[11]

As reported by the World Health Organization (WHO) in 2010, exposure to benzene has been related with a wide range of short-term and long-term health disorders.[12] Long-term benzene exposure is associated with suppression of bone marrow function, leading to a reduction in the number of red blood cells, white blood cells and its harmful impacts depends on the amount, route and duration of exposure, as well as the age and pre-existing medical condition of the exposed person.[13]

Evidence has shown that exposure to gasoline results in several health effects in humans, including decline in all blood cell formation, and increased risk of developing malignancies.[14] Studies on the blood cell parameters of gasoline station workers in countries have yielded contradictory results. Some studies have shown that there are no statistically significant differences between hematological parameters of people exposed to gasoline and the unexposed group in terms of red blood cell count, hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell count (WBC) and differential white blood cell count, and platelet count (PLT).[15] However, another study showed that RBC counts and Hct values were significantly higher in people exposed to gasoline than in the unexposed group.[16,17] Other studies have reported that the WBC count, RBC count, PLT counts, Hgb, Hct, and MCH values were significantly lower in the exposed group than in the unexposed groups.[18–20] Moreover, information on Ethiopia, particularly in the study area is limited. Results obtained from hematologic analyzers can provide change in hematological parameters such as red blood cell indices, WBC count and its differential, Hgb, Hct, PLT, mean platelet volume (MPV), and other parameters.[15,21] Studying these parameters helps study participants to understand the risk of occupational exposure on their hematological parameters and to take appropriate safety measures. Therefore, this study aimed to assess the hematological parameters of gasoline-exposed individuals in comparison with unexposed controls in Hossana Town, Southwest Ethiopia.

Methods and Materials

Study Design, Period and Area

A comparative cross-sectional study was conducted from May 01 to June 15, 2020, in Hossana Town, Southwest Ethiopia. Hossana town is the administrative center of the Hadiya zone in the south Ethiopia region, located 232 km away from the capital city of Ethiopia, Addis Ababa, and 157 km from the regional city of Hawassa, with a total population of 75,963. It has a latitude and longitude of 7°33′N 37°51′E with an elevation of 2177 meters above sea level. Five gas stations were located in the town of Hosanna, and 65 gasoline station workers were employed during the study period.

Study Participants

All adult workers at the five gas stations in Hossana town were recruited in the study. In addition, study participants working at Wachemo University Nigist Eleni Mohammed Memorial Teaching and Comprehensive Specialized Hospital (WUNEMMCSH) were included as controls. Study participants who had chronic diseases (cardiovascular, hematological malignancies, liver, and renal disease), participants who were on medication affecting blood cell count (Erythropoietin

therapy, hematin factors), participants with a history of blood transfusion in the last 3 months, pregnant women, and participants working in gasoline station for less than six months were not recruited in this study.

Sample Size Determination and Sampling Technique

One hundred and eighty study participants, comprising 60 gas station workers in the exposed group and 120 WUNEMMRTH staff members in the control group, were included. A total of 65 gasoline station workers worked in five substations in Hosanna town. Of the 65 gasoline station workers, 60 provided blood samples for analysis. Two gas station workers were not volunteers to participate, two were left out due to cardiovascular disease, and one pregnant mother. A convenience sampling technique was used to recruit staff members to the control groups.

Data Collection and Laboratory Methods

Socio-demographic and related data were collected using a pretested structured questionnaire via face-to-face interviews. After the interview, 4mL of venous blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes by laboratory professional. Complete blood counts (CBC) were performed using UniCel DxH 800 (BECKMAN COULTER, UniCel® DxH 800, USA) automated hematology analyzer. A peripheral blood film was prepared to investigate abnormalities in red blood cell, white blood cell, and platelet morphology of gasoline station workers. All laboratory tests were conducted at hematology laboratory of WUNEMMRH located in Hosanna Town.

Data Quality Assurance and Management

All quality assurance phases were considered to ensure data quality. The English version of the questionnaire was translated into the local language (Hadiyisa), translated back into English for accuracy and consistency. The questionnaire was pretested and data collectors were trained before actual data collection began. All blood samples were analyzed within two hours of collection. Manufacturer's instructions and standard operating procedures were strictly followed during sampling and experimental procedures. For automated hematology analyzer, background checks, repeated analysis of randomly selected samples to see reproducibility; randomly selected samples were verified using other similar hematology analyzer, and as part of the testing process, hospital laboratories evaluated the performance of the devices using the whole blood quality control.

Statistical Analysis and Interpretation

Data were cleaned and checked for its consistency and completeness. Then, the data entered into Epi-data version 3.1 (Epi-Data, Odense, Denmark) and exported into Statistical Package for Social Science (SPSS) version 25 (SPSS, Chicago, USA) statistical software for Windows. All continuous data were tested for normality by histogram and Shapiro–Wilk test. Hematological parameters were compared between gasoline-exposed workers and unexposed group using an independent-samples *t*-test for normally distributed data and the Mann–Whitney *U*-test for non-normally distributed data. One-way ANOVA was used for the comparison of hematological parameters according to duration of exposure. The results were summarized as mean ± standard deviation (SD), median, and interquartile range (IQR). P-value <0.05 was set as statistically significant for all analysis. The Hgb concentration was adjusted for altitude according to WHO standard guidelines to define anemia.

Ethical Consideration

This study was carried out according to the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of Jimma University, Institute of Health with protocol number of IRB000130/2020. Written permission was obtained from the director of WUNEMMRTH and Gasoline Station Owners. Each participant gave their written informed consent after they are fully informed about the study's objectives, methods, and purpose of the study. Participants in the study were identified by codes rather than by individual identifiers to ensure confidentiality. Any abnormal results were communicated to healthcare provider to manage them appropriately.

Results

Socio-Demographic Characteristics of Study Participants

One hundred eighty 180 (60 gasoline station workers and 120 control group) study participants were included in this study. Gasoline station workers and control groups showed significant differences in terms of sex and educational status ($p<0.05$). The mean age was 30±5.23 for gasoline station workers and 31±5.49 years for control group. Out of the total participants, 4 (6.70%) and 116 (96.70%) had a higher educational level for gasoline station workers and controls, respectively. Thirty-eight (63.30%) and 54 (45.00%) participants were single for gasoline station workers and controls, respectively (Table 1).

Comparison of Hematological Parameters Among the Study Participants

Statistically significant differences were observed in the median (IQR) of Hgb (P=0.001) and mean ± SD of RBC count (P=0.007) between gasoline station workers and control group. The median absolute eosinophil count (IQR) was significantly higher in gas station employees than in control groups (P=0.01). Levels of other hematological parameters, such as MCHC, RDW, and absolute monocyte count, were not significantly different between groups (Table 2).

Comparison of Hematological Parameters Based on Duration of Exposure

The mean of MCV was significantly decreased among participants with longer duration of work experience as compared to their counter parts (P=0.04). Other parameters varied non-significantly with years of working experience in gasoline station workers (Table 3).

Magnitude of Hematological Abnormality Among Gasoline Station Workers

The magnitudes of leukopenia, anemia, and thrombocytopenia among the gasoline-exposed workers were 4 (6.60%), 7 (11.70%), and 6(10.00%), respectively. On the other hand, among those gasoline station workers, 5 (8.30%) had leukocytosis. Thrombocytosis was not observed in gasoline station workers. In addition, all gasoline station workers were examined for peripheral morphology. Of the examined peripheral blood films, 49 (81.70%) of the result were normocytic normochromic RBCs, 7 (11.70%) had macrocytic oval shaped RBCs with increasing corresponding MCV values, and 4 (6.6%) study participant had microcytic RBCs with reduced MCV values (66 fL).

Table 1 Socio-Demographic Characteristics of the Study Group at Hossana Town, Southwest Ethiopia, from May 01 to June 15, 2020 (n=180)

Variables	Categories	Gasoline Station Workers (n=60)	Control Group (n=120)	X^2-test
Gender	Male n (%)	56 (93.30%)	92 (76.7%)	P=0.006*
	Female n (%)	4 (6.70%)	28 (23.3%)	
Age in years	20–24	15 (25.00%)	30 (25%)	P=0.700
	25–29	33 (55.00%)	58 (48.3%)	
	30–39	11 (18.30%)	30 (25%)	
	≥40	1 (1.70%)	2 (1.7%)	
Marital status	Single	38 (63.30%)	54 (45%)	P=0.500
	Married	20 (23.30%)	62 (51.7%)	
	Divorced	1 (1.70%)	1 (0.8%)	
	Widowed	1 (1.70%)	3 (2.5%)	
Educational status	No formal education	13 (21.70%)	–	P=0.01*
	Read and write	13 (21.70%)	–	
	Primary	22 (36.70%)	3 (2.5%)	
	Secondary	8 (13.30%)	1 (0.8%)	
	College/University	4 (6.70%)	116 (96.7%)	

Note: *P-value <0.05 is considered as statistically significant.

Table 2 Hematological Parameters of the Study Participants at Hossana Town, Southwest Ethiopia, from May 01 to June 15, 2020 (n=180)

Parameters	Gasoline Station Workers (n=60)	Control Group (n=120)	P-value
WBC ($\times10^3/\mu L$)[α]	7.31 ±2.39	7.00 ±1.98	0.39
RBC ($\times10^6/\mu L$)[α]	4.87 ±0.62	5.08 ±0.41	0.007*
Hgb (g/dl)	14.38 (1.70)	15.40 (1.30)	0.001*
HCT (%)	45.70 (5.80)	47.70 (6.50)	0.04*
MCV (fl)[α]	89.08 ±4.23	89.30 ±3.59	0.74
MCH (pg)	29.20 (2.07)	29.30 (1.60)	0.192
MCHC (g/dl)	32.90 (1.17)	32.90 (1.00)	0.82
RDW (%)[α]	13.80 ±0.65	13.80 ±0.70	0.62
PLT ($\times10^3/\mu L$)[α]	264.67 ±73.19	272.61 ±62.00	0.44
MPV (fl)[α]	7.903 ±0.75	7.83 ±0.60	0.78
Neutrophil absolute	4.20 (3.85)	4.30 (2.50)	0.58
Lymphocyte absolute	1.85 (1.17)	1.70 (1.00)	0.57
Monocyte absolute	0.50 (0.20)	0.50 (0.20)	0.16
Eosinophil absolute	0.57 (0.30)	0.30 (0.20)	0.01*
Basophil absolute	0.00 (0.10)	0.00 (0.10)	0.79
Neutrophil relative	58.40 (23.60)	63.60 (19.00)	0.66
Lymphocyte relative[α]	24.36 ±9.38	27.00 ±7.70	0.04*
Monocyte relative	6.60 (2.95)	6.65 (3.00)	0.61
Eosinophil relative	6.92 (3.40)	3.70 (2.45)	0.09
Basophil relative	0.57 (0.50)	0.60 ±0.50	0.17

Notes: [α]P-value derived from independent sample *T*-test, *p-value <0.05 is considered as statistically significant.
Abbreviations: WBC, White Blood Cell; RBC, Red Blood Cell; Hgb, Hemoglobin; HCT, Hematocrit; MCV, Mean Cell Volume; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; RDW, Red Cell Distribution Width; MPV, Mean Platelet Volume.

Table 3 Comparison of Hematological Parameters Based on Duration of Exposure Among Gasoline Station Workers at Hosanna Town, Southwest Ethiopia, from May 01 to June 15, 2020

Parameters	Duration of Exposure (in Year)			P-value
	1	2–5	>5	
WBC ($\times10^3/\mu L$) (Mean ±SD)	7.16±2.32	7.5±2.31	7.2±2.60	0.80
RBC ($\times10^6/\mu L$) (Mean ±SD)	5.09±0.65	4.8±0.50	4.75±0.66	0.20
MCV (fl) (Mean ±SD)	90±2.94	89.4±4.60	87.9±3.00	0.04*
RDW (%) (Mean ±SD)	13.67±0.65	13.83±0.50	13.88±0.78	0.58
LYM (%) (Mean ±SD)	26.23±9.90	27.65±8.20	21.46±9.60	0.31
PLT ($\times10^3/\mu L$) (Mean ±SD)	266.5±51	279.6±79	253±88.00	0.50
MPV (fL) (Mean ±SD)	8.1±0.78	7.79±0.69	7.8±0.79	0.37

Notes: *P-value <0.05 is considered as statistically significant.
Abbreviations: WBC, White Blood Cell; LYM%, Relative Lymphocyte; RBC, Red Blood Cell; MCV, Mean Cell Volume; RDW, Red Cell Distribution Width; MPV, Mean Platelet Volume; SD, standard deviation.

Discussion

Human exposure to benzene is associated with many adverse health effects, primarily related to the impairment of the hematopoietic system and bone marrow suppression. The present study showed that the levels of red blood cells, Hgb and Hct in gas station workers were significantly reduced compared to those in the control group. These are consistent with the results of other studies.[18,22,23] The decrease in Hgb and red blood cell count may be explained by the effect of metabolic end products and free radicals which shortened red blood cell lifespan. These free radicals can also alter

erythrocyte membrane and heme protein synthesis in bone marrow.[18,22,24] Moreover, the decline in these parameters might be due to the hematotoxic effect of benzene, which causes impairment of the hemopoietic system with bone marrow suppression leading to pancytopenia.[25]

The findings of the current study are in contrast to those of a study done in Iraq on 38 subjects who were exposed to benzene and control group. Between the benzene-exposed workers (filling workers) and the control group, there were no significant differences in the mean values of red blood cell count, Hgb, Hct, MCV, MCH, MCHC, RDW, white blood cell count, differential white blood cell count, or PLT count.[15] This might be due to differences in the duration of exposure and daily duty hours of the participants, as well as differences in the sample size of the study. The red cell indices of the present study are in line with those of a study conducted in Surat City on 30 gasoline station worker with ≥1 year of in duration of exposure, and 30 controls showed that there were no significant differences in red blood cell indices such as MCV and MCH between the exposed workers and control groups, which is similar to the present study.[26] Contrary to the study done by Pune, India, MCV and MCH were significantly higher in the exposed workers than in the non-exposed control group.[22] This might be due to differences in the length of exposure and the small size of previous studies.

Similar to our findings, the study in Bhubaneswar found no statistically significant difference in platelet count between the gasoline exposed and control group.[27] In contrast to current findings, UK study from Texas City found that participants exposed to benzene had a non-significantly higher platelet count.[16,20] According to the current study, gasoline station worker had a higher WBC count than the control groups, but it is not statistically significant. This outcome was in line with one from a study done in Nigeria.[28] Various studies could provide an explanation for this: high infection rates in groups that have been exposed because of the toxic gasoline products' immune-suppressing effects, which then cause WBC counts to rise.[29] On the other hand, a study conducted in India showed a decrease in WBC count between gasoline station workers and control groups.[30] This might be due to differences in the length of exposure time of the study participants.

In this study, MCV was significantly decreased with respect to the duration of exposure, whereas other parameters such as platelet count, relative lymphocyte count, and RDW varied insignificantly with respect to the duration of working experience in gasoline station workers. This finding is in agreement with a study conducted in the Dehradun region, where petrol pump workers with longer exposure times experienced more adverse effects on hematological parameters.[17]

The results of present study displayed the prevalence of hematological abnormalities, such as leukopenia (6.6%), anemia (11.7%), and thrombocytopenia (10%), among gasoline station workers. The hematopoietic system is extremely sensitive to the most volatile organic solvents that reach the bloodstream. These solvents may interfere with the process of RBC proliferation, causing adverse effects on heme synthesis and life spans of RBCs.[31] Another reason is that the toxic effects of prolonged exposure to benzene metabolites might cause suppression of the bone marrow, resulting in changes in blood parameters, which could cause anemia, leukopenia, or thrombocytopenia.[22] The prevalence of anemia in this study among those gasoline station workers was 11.7%, which is in line with EDHS report, of which 11% of men aged between 15–49 were anemic,[32] and lower than the EDHS report of 2016, 15% of men aged between 15 and 49 were anemic.[32]

Regarding the peripheral blood film, 6.6% microcytic red cells with reduced MCV values gasoline station workers, which is inconsistent with study conducted in Sudan[8] from 2014 to 2015 reported regarding red blood cell morphology 50% of the gasoline station workers showed a microcytic picture. This inconsistency might be differences in the duration of exposure and sample size of the study. The current study revealed that macrocytosis of red cell morphology occurred in 11.7% of cases, which is consistent with research from Nigeria and Iran.[33,34] This macrocytosis might be due to the toxic component of gasoline, which can affect erythropoiesis, alter the RBC membrane, make it more susceptible to hemolysis, obstruct replication, and cause macrocytic anemia.[22]

Conclusion and Recommendation

This study showed that some hematological parameters are changed in gasoline station workers as compared to the control groups. These hematological alterations include decreased RBC, Hgb, and Hct counts and increased absolute eosinophil counts in gasoline station workers than control group. This study therefore came to the conclusion that there might be link between exposure to gasoline and hematological changes. Medical observations, including prior to

employment and periodic medical check-ups of the hematological profile before the development of chronic impairment should be considered. Further long-term prospective study with an increased sample size of gasoline workers is needed to obtain a more complete picture of the long-term effects and define effects of a single chemical to which gasoline station workers are exposed.

Data Sharing Statement

This paper is based on the thesis of Tamirat Ersino. It has been published on Jimma university website, which is available https://repository.ju.edu.et/bitstream/handle/123456789/4204/TAMIRAT-ERSINO%20final%20thesis.pdf?isAllowed=y&sequence=1.[2] All relevant data related to this work are included within the manuscript.

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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 drafting, revising, and critically reviewing the manuscript. All authors have agreed on approval of the final manuscript to be published in the current journal and to be accountable for all aspects of the work.

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Disclosure

The authors declare that there is no conflict of interest for this work.

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CASE REPORT

Severe Cytokine Release Syndrome and Hemophagocytic Lymphohistiocytosis (HLH)-Like Syndrome Following Administration of Combined Brentuximab Vedotin and Nivolumab for Recurrent Classical Hodgkin Lymphoma: A Case Report

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Abstract: Brentuximab vedotin (BV) and nivolumab are increasingly utilized as a novel regimen in patients with relapsed/refractory classical Hodgkin lymphoma (cHL). A 26-year-old male presented to the hospital with refractory diabetic ketoacidosis and multiple electrolyte abnormalities, 9 days after the first dose of brentuximab vedotin and nivolumab for recurrent classical Hodgkin lymphoma. During his hospitalization, he developed multi-organ failure. His workup showed elevated cytokine levels concerning severe cytokine release syndrome (CRS) and hemophagocytic lymphohistiocytosis (HLH)-like syndrome. Despite treatment with CRS- and HLH-directed therapies, his clinical status deteriorated due to ongoing multifactorial shock and worsening multi-organ dysfunction, and comfort care measures were eventually pursued. To our knowledge, there have been no other cases reported of HLH-like syndrome after the combination of BV and nivolumab in patients with cHL. This case of a fatal adverse event following one dose of BV and nivolumab underscores the vital need for close monitoring of patients receiving this treatment regimen.

Keywords: immunotherapy, immune-related adverse event, antibody drug conjugate, CRS

Introduction

The combination of brentuximab vedotin (BV) and nivolumab has emerged as a compelling regimen with a good safety profile for older or unfit patients with classical Hodgkin lymphoma (cHL) who cannot tolerate intensive chemotherapy and patients with relapsed/refractory (R/R) cHL. BV plus nivolumab is therefore now utilized as a therapeutic option in R/R cHL.[1–4] Commonly associated toxicities include peripheral neuropathy and immune-related adverse events such as rash and endocrinopathies. Here, we describe a fatal adverse event following the first dose of BV and nivolumab in a young patient, highlighting the need for careful safety monitoring with the use of this regimen.

Case Report

A 21-year-old Caucasian male was diagnosed with stage IIB unfavorable-risk cHL, nodular sclerosing type, and achieved a complete response (CR) after four cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) followed by involved-site radiation therapy (ISRT) targeting disease in the chest. Five years later, surveillance positron emission tomography-computed tomography (PET-CT) revealed hypermetabolic anterior mediastinal soft tissue masses involving the left side of the sternum and left pectoral muscle as well as multiple hypermetabolic lymph nodes (Deauville score of 5); no disease was noted elsewhere (Figure 1A). A biopsy of one of the chest wall masses confirmed recurrent cHL. As

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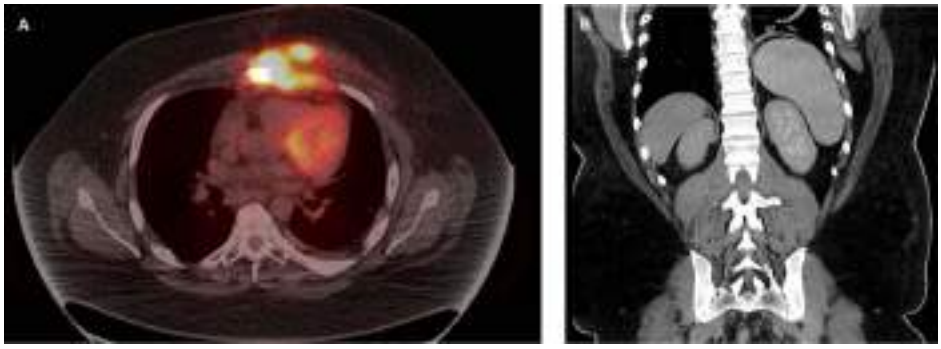

Figure 1 (**A**) Pre-treatment PET-CT. (**B**) Post-cycle 1 CT abdomen showing splenomegaly.

he had no significant history of autoimmune disease other than atopic dermatitis, he was a candidate for immunotherapy. He was recommended to complete 4–6 cycles of BV plus nivolumab followed by consolidation with high-dose chemotherapy and autologous stem cell transplantation. Baseline blood counts prior to starting treatment revealed mild anemia with hemoglobin of 11.5 g/dL (normal, 13.2–16.6), normal platelet count of 351×10(9)/L (normal, 150–450×10(9)/L), and normal white blood cell count (WBC) of 8×10(9)/L (normal, 4–10×10(9)/L). Comprehensive metabolic panel showed mild hyponatremia of 134 mmol/L (normal, 135–145 mmol/L), normal creatinine of 0.84 mg/dL, normal glucose of 91 mg/dL (normal, 70–140 mg/dL), and normal liver enzymes. The patient started treatment with BV (1.8 mg/kg) and nivolumab (3 mg/kg).

Nine days after the first dose, he presented to the hospital with diffuse myalgias, back and abdominal pain, nausea, vomiting, dehydration, and low-grade fever (38°C), the latter of which had started approximately 5 days prior to his hospitalization. On admission, he was afebrile and normotensive with blood pressure of 128/83 but tachycardic to 130 bpm and mildly tachypneic. Physical examination showed mild right-sided abdominal tenderness without rebound or guarding. Laboratory testing was consistent with diabetic ketoacidosis (DKA) based on hyperglycemia with serum glucose level of 459 mg/dL, anion-gap metabolic acidosis with elevated lactate level of 4.5 mmol/L, and ketonuria. He also had grade 1 transaminitis with alanine aminotransferase (ALT) 87 U/L (normal, 7–55 U/L) and aspartate aminotransferase (AST) 105 U/L (normal, 8–48 U/L). Admission blood counts included hemoglobin 10.4 g/dL, new-onset thrombocytopenia with platelet count of 133×10(9)/L, and normal WBC, 5.8×10(9)/L. Coagulation studies were not available on admission. Computed tomography (CT) scan of the abdomen and pelvis was notable for splenomegaly measuring 16 cm that was not present on pre-treatment PET-CT scan (Figure 1B).

Despite initiation of appropriate DKA treatment, the patient's acidosis worsened. He developed respiratory distress, distributive shock, and multiorgan dysfunction with acute renal injury and worsening transaminitis, necessitating transfer to the intensive care unit for intubation, vasopressor support, and continuous renal replacement therapy. In addition to transaminitis and splenomegaly, he was found to have several laboratory abnormalities suspicious for cytokine release syndrome (CRS) and hemophagocytic lymphohistiocytosis (HLH)-like syndrome, including hyperferritinemia, hypertriglyceridemia, and pancytopenia (Table 1). The patient's HScore was 181 points, correlating to a high score with 70–80% probability of hemophagocytic syndrome.

Although he was treated empirically with broad-spectrum antibiotics, extensive infectious workup including bacterial, fungal, and viral cultures was negative. Specifically, viral evaluation was negative for viral hepatitis, influenza, respiratory syncytial virus, cytomegalovirus, Epstein Barr virus, and herpes simplex virus. HIV, adenovirus, enterovirus, HHV-6, and parvovirus B19 testing were not performed. Pneumocystis jiroveci pneumonia smear was negative. A repeat CT scan of the chest, abdomen, and pelvis showed no acute pathology, redemonstrating splenomegaly and the known mediastinal mass. A biopsy to identify tissue evidence of hemophagocytosis could not be obtained due to the acuity of the patient's condition.

While awaiting the results of confirmatory HLH testing, the patient was started empirically on tocilizumab, etoposide, and glucocorticoids for treatment of both CRS and HLH. He also received therapeutic plasma exchange for management

Table 1 Laboratory Results Consistent with the Diagnosis of CRS and HLH-Like Syndrome

Labs	Laboratory Values	References
Ferritin	10,864 (prior to tocilizumab and etoposide) 6628 (after 1 dose of tocilizumab) 19,627 (after second dose of tocilizumab and 1 dose of etoposide)	24–336 mcg/L
Triglycerides	1366	<150 mg/dl
IL-9	28	<10 pg/mL
IL-6	>315	<5 pg/mL
IL-2 receptor alpha soluble (soluble CD-25)	>4000	<959 pg/mL
IL-18	2592	<468 pg/mL
Hemoglobin	9.2	13–15 gm/dl
Platelets	114	150–350 $x10^9$/L
Absolute neutrophil count	1.14	1.56–6.45x10 (9)/L
ALT	66	7–55 U/L
AST	193	8–48 U/L
Fibrinogen	149	200–393 mg/dL

Abbreviations: IL, Interleukin; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

of hypertriglyceridemia. Cytokine panel results did eventually show elevated soluble IL-2 receptor alpha of >4000 pg/mL (normal, <959 pg/mL), elevated IL-18 of 2592 pm/mL (normal, <468 pg/mL) and elevated IL-6 level of >315 pg/mL (normal, <5.0 pg/mL). Unfortunately, the patient's condition deteriorated as vasopressor requirements increased. Life support measures were withdrawn 2 days later in accordance with the patient's previously stated wishes. On autopsy, there was no clear tissue evidence of hemophagocytes. Figure 2 illustrates the case timeline.

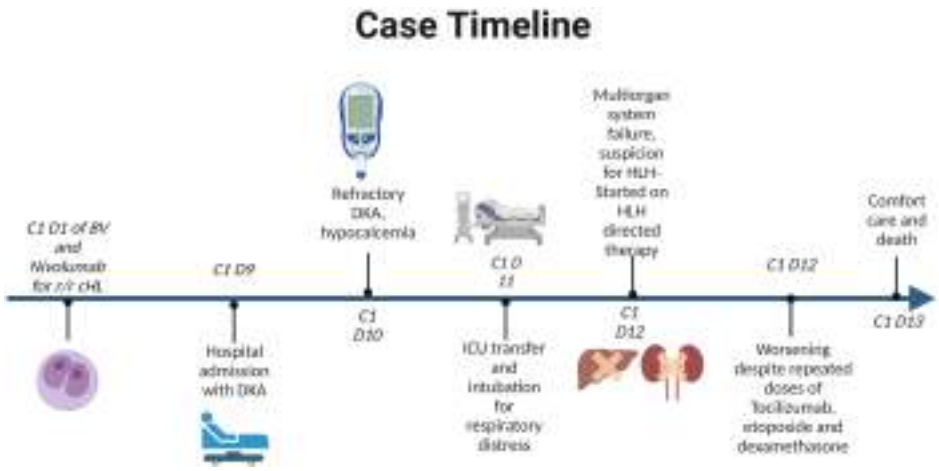

Figure 2 Timeline of the case.
Notes: Timeline created with BioRender.com.

Discussion

Our case report describes a fatal case of severe CRS and HLH-like syndrome after the first dose of BV and nivolumab, generally thought to be a well-tolerated and safe regimen.

Immune evasion has been identified as the hallmark of carcinogenesis, and immunotherapy has revolutionized cancer therapies by enhancing the immune system and identifying cancer cells as "non-self".[5] Immune checkpoint inhibitors (ICIs) became a standard of care for many solid cancers and hematological malignancies (ie, melanoma, renal cell carcinoma, non-small cell lung carcinoma, and Hodgkin lymphoma).[6]

However, as ICIs activate the T-cell-mediated immune response, active T-cells can attack the healthy tissues due to cross-reactivity between the T-cells of the tumor and normal tissues.[7] This phenomenon can give rise to a broad spectrum of immune-related adverse events (irAEs), of which the most common are endocrinopathies, gastrointestinal toxicity, pulmonary toxicity, arthritis, and skin rash.[8]

HLH is a severe, life-threatening disorder and has been rarely reported as an irAE secondary to ICIs. The pathophysiology of HLH with ICIs could be explained by immune system hyperactivation, which subsequently leads to increased levels of inflammatory cytokines and activation of the macrophages and reticuloendothelial system.[9] In our literature search, we found 61 studies on PubMed and 17 studies across Embase database reporting secondary HLH after using ICIs. The most common malignancy associated with HLH was malignant melanoma, followed by non-small cell lung carcinoma.[10]

Pembrolizumab is the most frequently reported ICI causing HLH followed by nivolumab or nivolumab/ipilimumab.[10] Moreover, Noseda et al reviewed the World Health Organization (WHO) global database of suspected drug reactions as of 2018; among 50,000 reported AEs from ICIs, HLH was reported in only 38 cases.[11] The time from the initiation of an ICI to the development of HLH is highly variable across reports. While HLH mainly occurs after prolonged exposure to ICIs, with a median duration of ICI treatment of 9.9 months, it can arise at any point during the treatment, ranging from a few days following exposure to ICIs to 17 months after treatment initiation.[10–13]

BV is an antibody–drug conjugate that targets CD30 chimeric IgG1 antibodies and disrupts the microtubule network, leading to cell death.[14] In 2018, the Food and Drug Administration (FDA) approved BV for use in the treatment of CD30-positive peripheral T-cell lymphomas (PTCL) based on the results of the ECHELON-2 trial, in which the most common AEs reported were peripheral neuropathy, GI toxicity, and myelosuppression.[15] Cytokine release syndrome (CRS) has rarely been reported in the literature in association with the use of BV; in 2015, Alig et al reported the first case of CRS after the first dose of BV in a patient with anaplastic large cell lymphoma (ALCL).[16] The activated T-cell response with BV can result in altered cytokine levels with increased levels of proinflammatory cytokines.[15] Among 7542 AEs reported for BV in the FDA adverse events reporting system (FAERS) database, 72 were for hyperglycemia, 32 for HLH, and 13 for CRS.[17] Although FAERS reported CRS and HLH with BV use, those incidents were not described previously on PubMed or Embase, making it impossible to determine whether these cases were genuinely associated with exposure to BV.

Distinguishing between primary HLH, secondary HLH, and CRS is challenging. CRS features can overlap with HLH.[18] Both CRS and HLH are characterized by a hyperinflammatory state and cytokine storm. Severe forms of CRS can progress to organ failure and HLH-like presentation. Due to this hyperinflammatory state, the cytokine profile in severe CRS can be very similar to the one seen in true HLH.[19] The presence of hepatosplenomegaly and histopathological evidence of hemophagocytosis would support the diagnosis of HLH and help distinguish between CRS and HLH. In our case, a tissue biopsy could not be pursued due to the patient's hemodynamic instability. The patient did meet the HLH-2004 criteria.[20] An autopsy showed splenomegaly with no definitive evidence of hemophagocytosis. We did not obtain an HLH gene panel, which would have been helpful to rule out genetic susceptibility to primary, adult-onset HLH and distinguish between primary and secondary HLH. Regardless, such testing can take several days to yield a result and would not have changed our treatment strategy in an acute setting. There is no history to suggest presence of familial HLH syndrome.

Our patient had evidence of hyperinflammatory state, CRS, and HLH-like syndrome with fever, splenomegaly, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, and elevated soluble IL-2 receptor alpha.[21] Other symptoms were consistent with treatment-related AEs, including refractory DKA, which can occur with exposure

to ICIs or BVs.[22–24] It is unclear whether BV, nivolumab, or the combination triggered the hyperinflammatory response in our patient. Moreover, we cannot exclude progression of cHL, given the new splenomegaly found on the patient's admission, as a contributor to the HLH-like presentation. It could be posited that DKA itself might have contributed to HLH-like syndrome, but this possibility is unlikely as the patient's clinical status worsened even after resolution of DKA. We suspect that the synergistic effect of BV and ICI on the immune system resulted in hyperactivation of immune-mediated cytokines and, ultimately, HLH-like syndrome.

Conclusion

With increasing applications of immunotherapeutic and targeted agents in many types of cancer, evidence continues to emerge about the potential toxicities of these agents. We aim to raise awareness among physicians and other medical providers about the possibility that even young patients receiving BV and nivolumab, generally regarded as a reasonably safe therapeutic combination, could develop life-threatening CRS and HLH-like syndrome. While ICIs have been linked to HLH previously, to our knowledge this case is the first in the literature to demonstrate a fatal event of HLH-like syndrome in cHL after the use of a combination of antibody–drug conjugate and ICI.

Ethics and Consent

The patient provided written informed consent for publication of the case report. Institutional approval was not required to publish the case details.

Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Prognostic Significance of Dual-Specificity Phosphatase 23 Expression in Acute Myeloid Leukemia

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Background: Recently, an increasing number of studies have suggested dual-specificity phosphatase 23 (DUSP23) is a critical factor in the development of diffuse connective tissue disease and may be a valuable biomarker for primary human cancers. However, there is a lack of comprehensive studies on the prognostic significance of DUSP23 expression in acute myeloid leukemia (AML).

Methods: RNA sequencing data from The Cancer Genome Atlas (TCGA) (AML = 173), Genotype-Tissue Expression (GTEx) (healthy controls = 70) and GEO (AML = 461, healthy controls = 76) databases were used to compare DUSP23 expression between AML patients and healthy controls. The overall survival (OS) of DUSP23 in AML was evaluated using Kaplan-Meier Cox regression. Furthermore, univariate Cox regression and multivariate Cox regression analysis were used to determine whether DUSP23 was an independent prognostic factor for AML. We then verified the expression level and prognostic significance of DUSP23 in our cohort (AML = 128, healthy controls = 31). In addition, functional enrichment analysis of DUSP23-related DEGs was performed through gene set enrichment analysis (GSEA) and protein-protein interaction (PPI) network analysis.

Results: The expression level of DUSP23 is significantly higher in AML patients than in healthy controls in TCGA, GTEx, GEO databases and our cohort. By multivariate analysis, high expression of DUSP23 is a poor prognostic indicator of OS in the TCGA database. Next, we verified the role of DUSP23 as an adverse prognostic biomarker in our cohort. Enrichment analysis of related genes showed that DUSP23 may regulate important signal pathways in hematological tumors including the MAPK pathways. It is suggested by the PPI network that DUSP23, along with IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D may play a role in the process of AML.

Conclusion: The study demonstrated high expression of DUSP23 could serve as a poor independent prognostic biomarker in AML.

Keywords: DUSP23, expression, prognosis, AML, bioinformatics

Introduction

AML is highly heterogeneous and represents a cohort of clonal malignancies derived from myeloid precursors.[1] Existing drugs' poor therapeutic effects are attributed to leukemia's complex molecular mechanisms and chemoresistance.[2] The molecular basis of AML, aided by biomarkers, can play an essential role in AML diagnosis, prognostic stratification, residual leukemia monitoring, treatment response prediction, and targeted drug development. Therefore, it is necessary to discover novel biomarkers to better understand the molecular basis of AML.[3,4]

Homoharringtonine (HHT) is widely used in clinical practice. Because of its anti-leukemic efficacy, cost-effectiveness, and minimal toxicity. HHT-resistant strains were constructed by our research team to explore the mechanism of HHT resistance. It was observed that the DUSP23 gene exhibited differential low expression in HHT-resistant cells than sensitive cells.[5] This indicated that DUSP23 may serve as a therapeutic target and a potential prognostic factor in AML. DUSP23 is located on chromosome 1q23 and is characterized by a dual-specificity phosphatase catalytic domain.[6] It belongs to the low-molecular-weight VHR-like subfamily of the dual-specificity phosphatase family. The expression of DUSP23 is tissue-specific, being expressed only in a few

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normal tissues but more in fetal and tumor tissues, suggesting that its function may be related to embryonic development and tumor growth.[7] So far, the expression of DUSP23 in AML and its prognostic value remain unclear.

The objective of this study was to assess the prognostic significance of DUSP23 in AML. Firstly, RNA sequencing data of AML patients and healthy controls were obtained from the TCGA, GTEx and GEO databases to examine the expression characteristics of DUSP23. Subsequently, the clinical significance of DUSP23 in AML was assessed by using Kaplan-Meier and Cox regression analyses and developing a nomogram prognostic model. Next, we verified the differences in DUSP23 expression between AML patients and healthy controls in our cohort and assessed its prognostic relevance. Additionally, functional enrichment analyses of DUSP23 were conducted using various methods, including GO, KEGG, GSEA, the PPI network, SSGSEA, and immune cell infiltration analysis, to provide a preliminary grasp of the underlying molecular mechanisms of DUSP23 in AML carcinogenesis and progression. Based on the above results, the prognostic value of DUSP23 for AML was confirmed.

Materials and Methods

Data Source

RNA-seq data and clinical information of AML patients for the TCGA and GTEx databases were obtained from the UCSC XENA browser (https://portal.gdc.cancer.gov;https://xenabrowser.net/datapages/),[8–12] and the RNA-seq dataset GSE6891 was downloaded from the GEO databases https://www.ncbi.nlm.nih.gov/geo/).[13–15] The TCGA databases include 173 AML patient samples, the GTEx databases include 70 healthy controls. The GSE6891 dataset, derived from the GPL570 platform, comprises 461 AML patient samples and 76 healthy controls. TPM values obtained from RNA-Seq were utilized, and a log2 transformation was performed for intrasample comparisons to ensure data consistency across different databases. Samples without survival data were excluded from the analysis.

Patients' Samples

A total of 128 AML patients and 31 healthy control bone marrow samples were collected at the Affiliated People's Hospital of Ningbo University. Written informed consent was obtained from all participants before their involvement in the study. This study was approved by the Research Ethics Committee of the Affiliated People's Hospital of Ningbo University. Patients were divided into two groups based on the cut-off value of DUSP23 mRNA expression. Non-M3 patients received standard anthracycline plus cytarabine therapy, while M3 patients received all-trans retinoic acid (ATRA) plus arsenic trioxide (ATO) therapy. Baseline clinical characteristics are shown in Table 1.

Table 1 Characteristics of AML Patients by High and Low DUSP23 Expression

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
n		71	37	
Age, n (%)	<= 60	30(42.3%)	22(59.5%)	0.089
	> 60	41(57.7%)	15(40.5%)	
WBC count(x10^9/L), n (%)	<= 20	32(37.5)	25(67.6%)	0.026
	> 20	39(54.9%)	12(32.4%)	
BM blasts (%), n (%)	<= 20	5(7%)	3(8.1%)	0.841
	> 20	66(93%)	34(91.9%)	
Gender, n (%)	Male	42(59.2%)	20(54.1%)	0.611
	Female	29(40.8%)	17(45.9%)	
FLT3 mutation, n (%)	Negative	59(83.1%)	28(75.7%)	0.355
	Positive	12(16.9%)	9(24.3%)	
DNMT3A, n(%)	Negative	61(85.9%)	33(89.2%)	0.858
	Positive	10(14.1%)	4(10.8%)	

(*Continued*)

Table 1 (Continued).

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
KIT, n(%)	Negative	61(85.9%)	34(91.9%)	0.552
	Positive	10(14.1%)	3(8.1%)	
NPM1 mutation, n(%)	Negative	53(74.6%)	33(89.2%)	0.126
	Positive	18(25.4%)	4(10.8%)	
Cytogenetics, n (%)	t(8;21)	5 (55.6%)	5(71.4%)	0.672
	inv(16)	3(33.3%)	1(14.3%)	
	t(9;22)	1(11.1%)	1 (14.3%)	
Consolidation, n(%)	HSCT	24(33.8%)	9(24.3%)	0.31
	Chemotherapy	47(66.2%)	28(75.7%)	

Validation of Prognostic Gene by Quantitative Real-Time PCR (qRT-PCR) Analysis

RNA extraction from the tissue samples was conducted using Trizol reagent (Invitrogen, Shanghai, China) following the manufacturer's protocol. The obtained RNA (1000 ng) was then utilized for cDNA synthesis through a cDNA synthesis kit (Takara, Tokyo, Japan). qRT-PCR was performed using TB Green PCR Master Mix (Takara, Tokyo, Japan) on a 7500 Fast Real-Time PCR System (Applied Biosystems, Singapore), and the relative expression of DUSP23 was determined using the comparative cycle threshold (Ct) method.[16,17] The 2–ΔΔCt method was employed to assess gene expression relative to glyceraldehyde—3—phosphate dehydrogenase (GAPDH) for AML cells. The primers were as follows: DUSP23 forward: 5′-GCCATTGCTGAAATCCGACG-3′; reverse:5′-CTGCTCATAGGTCTCGATGGA-3′; GAPDH forward: 5′-ATGGGGAAGGTGAAGGTCG-3′; reverse: 5′-GGGTCATTGATGGCAACAATATCCATA-3′.

Prognostic Model Generation and Prediction

A nomogram was created using the RMS R package (version 6.3–0) to personalize the prediction of OS in AML patients, incorporating significant clinical characteristics and calibration plots. Calibration curves were used to assess the accuracy of the nomogram, which compared the observed rates with the nomogram-predicted probabilities. The optimal predictive values were represented by the 45° line. The discrimination ability of the nomogram was determined by the concordance index (C-index), which was computed using 8000 bootstraps resamples.[18]

DEG Analysis

The expression profile of high- and low-expression levels of DUSP23 (cut-off value of 50%) in AML samples (HTseq-Count) were compared using the DESeq2 R package to identify DEGs.[19,20] A heatmap was used to visualize the top 20 DEGs (Figure 1B).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichments Analysis of DEGs

A threshold of |logFC| >1.5 and padj<0.05 was applied to select DEGs for functional enrichment analysis, and R (4.2.1) was used for this analysis. The clustered DEGs were visualized as a heatmap using ggplot2[3.3.6] in R.

GSEA

GSEA was performed using the R (4.2.1) to identify functional and pathway differences between the high- and low-expression groups of DUSP23. Significance was determined based on an adjusted P-value < 0.05 and FDR q-value < 0.25.

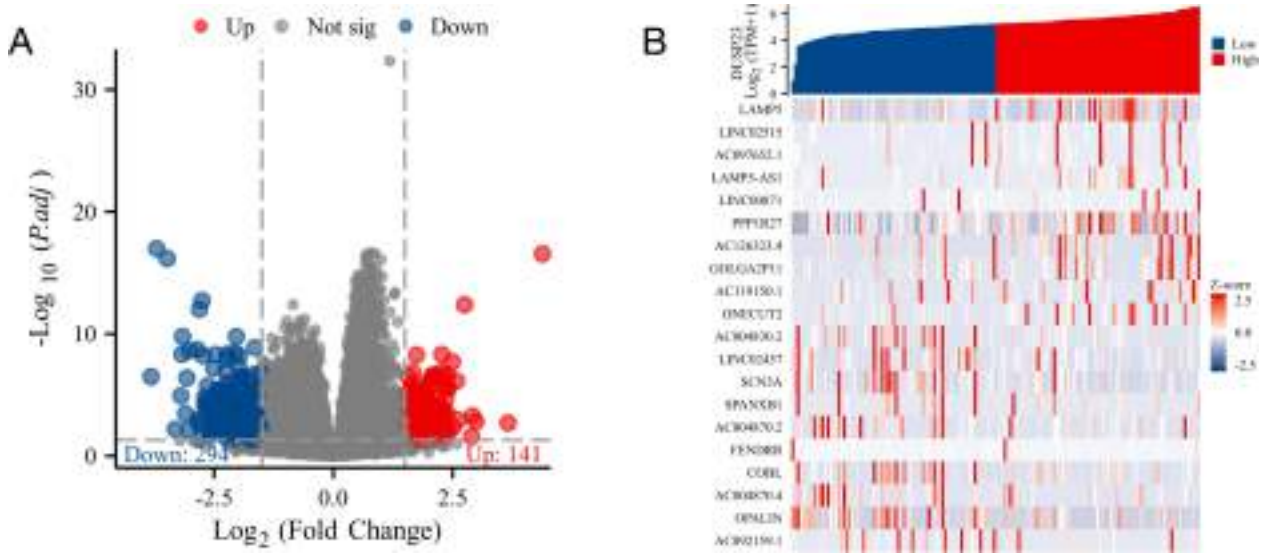

Figure 1 DEGs of the high- and low-DUSP23 expression groups in AML. (**A**) Volcano plot of DUSP23-related DEGs. (**B**) Heatmap of DUSP23-related DEGs.

PPI Network

The PPI network was derived from the STRING database (https://string-db.org/) to estimate the interactional correlations of the DEGs.[21] A confidence score >0.45 was considered significant. Hub proteins and key nodes in the constructed PPI network were identified using the Cytoscape plugin CytoHubba.[5,22]

Immune Infiltration Analysis

SSGSEA analysis was performed using the GSVA package in R (version 3.6.3) to analyze immune infiltration in DUSP23. Data on 24 types of infiltrating immune cells, as previously described, were obtained. The correlation between DUSP23 expression and the enrichment scores of the 24 types of immune cells was assessed using Spearman correlation.[23,24] Moreover, the enrichment scores between high- and low-DUSP23 expression groups were compared using the Wilcoxon rank-sum test.

Statistical Analysis

The statistical data obtained from TCGA and GTEX were analyzed using R-4.2.1. Prognostic factors were evaluated using Cox regression analysis and the Kaplan-Meier method. The median expression of DUSP23 was taken as the cut-off value. A statistically significant result was considered as a P-value less than 0.05 in all tests. Furthermore, the effectiveness of the transcriptional expression of DUSP23 in differentiating AML from healthy samples was assessed using ROC analysis on the pROC package. The area under the curve (AUC) value, computed in the range of 0.5 to 1.0, indicated a discrimination ability of 50–100%. OS is defined as the length of time from a specific point in time, such as diagnosis or treatment, that patients with a disease are still alive.

Results

DUSP23 Expression in Pan-Cancer and AML

In pan-cancer analysis, the expression profiles of DUSP23 in normal and malignant samples were determined. Remarkably, DUSP23 was upregulated in multiple malignancies like Bladder cancer, ovarian cancer, etc. (Supplementary Figure 1). In addition, DUSP23 was also abnormally highly expressed in AML compared with healthy controls (Figure 2A). Subsequently, the result from GSE6891 also confirmed that DUSP23 expression is higher in AML patient samples (Figure 2B). To further investigate the significance of DUSP23 expression, the Wilcoxon Rank Sum test was used to compare its expression levels in patients with various clinicopathological features. The results indicated that DUSP23 was significantly upregulated in patients with > 70% peripheral blood (PB) blasts ($P < 0.05$) than those with ≤

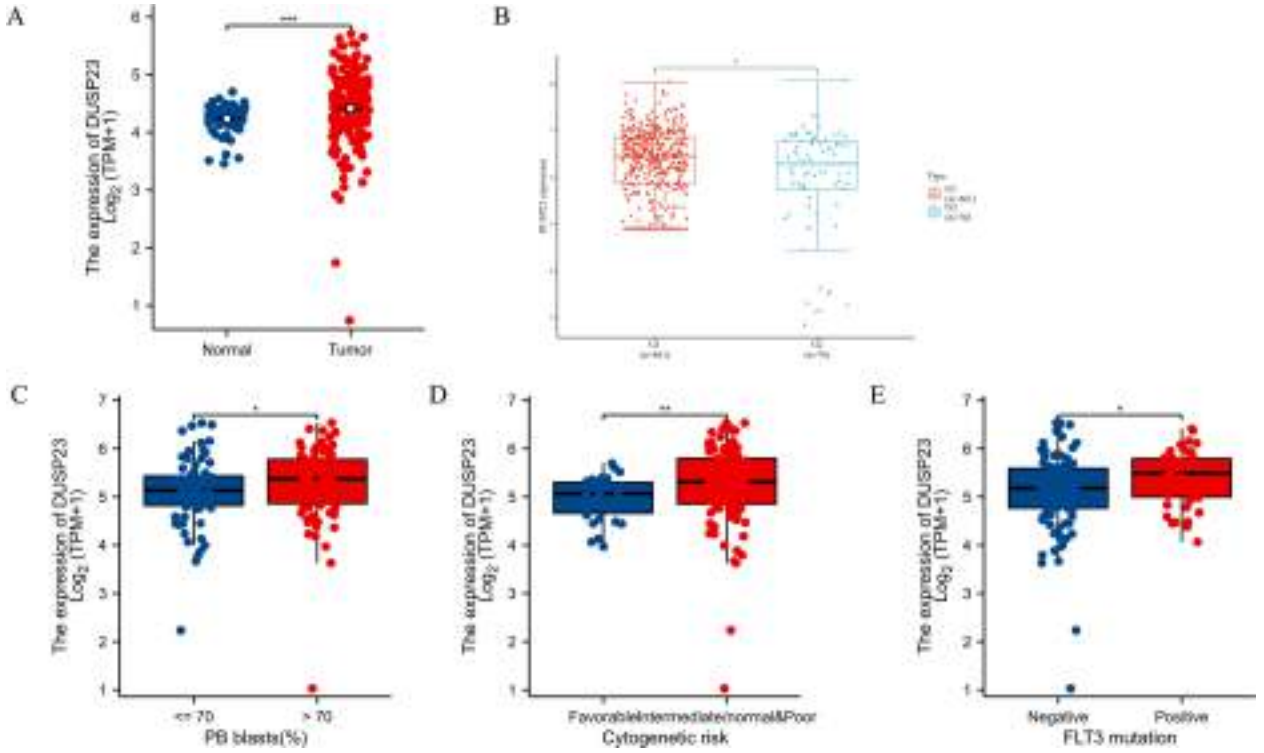

Figure 2 The association of DUSP23 expression with clinical characteristics. (**A**) Expression level of DUSP23 in paired normal and AML samples. Analysis between two groups: Wilcoxon Rank sum test; NS: P 0.05 or higher; (*P < 0.05; **P < 0.01; ***P < 0.001). (**B**) DUSP23 expression in AML from GEO databases (GSE6891). (**C**) PB blasts abundance, (**D**) Cytogenetic risk, (**E**) FLT3 mutation.

70% PB blasts, in patients with poor cytogenetic risk (P < 0.01) than intermediate/normal risk, and in FLT3 mutation-positive cases (P < 0.05) than FLT3 mutation-negative cases (Figure 2C–E). This may indicate that high DUSP23 expression is associated with an unfavorable prognosis.

The Relationship Between the Expression of DUSP23 and the Prognosis of AML Patients

The above results suggest that elevated expression of DUSP23 is associated with an unfavorable prognosis. Subsequently, the verification of whether DUSP23 is an independent predictive factor for unfavorable AML prognosis will be conducted. The clinical characteristics of TCGA patients were downloaded and divided into high and low expression groups according to the median of the expression of DUSP23 (Table 2). Notably, patients with higher DUSP23 expression had significantly higher WBC counts and PB blasts than those with lower DUSP23 expression (P = 0.017 and = 0.014, respectively; Table 2). There were also significant differences between the two groups in the incidence of each FAB classification and cytogenetic risk (P = 0.006 and = 0.016, respectively; Table 2). Higher DUSP23 expression was significantly correlated with FLT3 (P = 0.01; Table 2).

The study identified an association between poor OS and increased expression of DUSP23 in AML patients (Figure 3A). The robust prognostic value of DUSP23 for OS in AML patients was confirmed by ROC analysis, with a C-index of 0.647 (95% confidence interval: 0.580–0.714) (Figure 3B). In addition, cytogenetic risk (intermediate/normal and poor) and DUSP23 were also poor prognostic factors for AML patients (Figure 3C).

In addition, univariate Cox proportional hazards regression was used to assess factors influencing OS. The results revealed that high expression of DUSP23 (P = 0.009), poor & intermediate cytogenetic risk (P < 0.001), and age > 60 (P < 0.001) were predictive factors for worse OS (Table 3). Subsequently, multivariate Cox regression analysis including

Table 2 Association Between DUSP23 Expression and Clinicopathologic Features in AML Samples from the TCGA

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
n		75	75	
Age, n (%)	<= 60	45 (30%)	42 (28%)	0.620
	> 60	30 (20%)	33 (22%)	
WBC count (x10^9/L), n (%)	<= 20	45 (30.2%)	31 (20.8%)	0.017
	> 20	29 (19.5%)	44 (29.5%)	
BM blasts (%), n (%)	<= 20	32 (21.3%)	27 (18%)	0.403
	> 20	43 (28.7%)	48 (32%)	
PB blasts (%), n (%)	<= 70	43 (28.7%)	28 (18.7%)	0.014
	> 70	32 (21.3%)	47 (31.3%)	
Cytogenetic risk, n (%)	Favorable	22 (14.9%)	8 (5.4%)	0.016
	Intermediate/normal	35 (23.6%)	47 (31.8%)	
	Poor	18 (12.2%)	18 (12.2%)	
Gender, n (%)	Female	31 (20.7%)	36 (24%)	0.412
	Male	44 (29.3%)	39 (26%)	
Race, n (%)	Asian	1 (0.7%)	0 (0%)	0.670
	White	66 (44.3%)	69 (46.3%)	
	Black or African American	7 (4.7%)	6 (4%)	
FLT3 mutation, n (%)	Negative	57 (39%)	44 (30.1%)	0.010
	Positive	15 (10.3%)	30 (20.5%)	
IDH1 R132 mutation, n (%)	Negative	70 (47.3%)	65 (43.9%)	0.147
	Positive	4 (2.7%)	9 (6.1%)	
IDH1 R140 mutation, n (%)	Negative	67 (45.3%)	69 (46.6%)	0.547
	Positive	7 (4.7%)	5 (3.4%)	
IDH1 R172 mutation, n (%)	Negative	73 (49.3%)	73 (49.3%)	1.000
	Positive	1 (0.7%)	1 (0.7%)	
RAS mutation, n (%)	Negative	70 (47%)	71 (47.7%)	1.000
	Positive	4 (2.7%)	4 (2.7%)	
NPM1 mutation, n (%)	Negative	65 (43.6%)	51 (34.2%)	0.004
	Positive	9 (6%)	24 (16.1%)	
FAB classifications, n (%)	M0	11 (7.5%)	4 (2.7%)	0.006
	M1	11 (7.5%)	24 (16.4%)	
	M2	23 (15.8%)	15 (10.3%)	
	M3	9 (6.2%)	5 (3.4%)	
	M4	17 (11.6%)	12 (8.2%)	
	M5	3 (2.1%)	12 (8.2%)	
	M6	1 (0.7%)	1 (0.7%)	
	M7	0 (0%)	1 (0.7%)	
Cytogenetics, n (%)	+8	5 (4%)	3 (2.4%)	0.551
	Complex	13 (10.4%)	11 (8.8%)	
	del(7)	4 (3.2%)	2 (1.6%)	
	inv(16)	4 (3.2%)	4 (3.2%)	
	Normal	28 (22.4%)	41 (32.8%)	
	t(15;17)	6 (4.8%)	4 (3.2%)	

age, cytogenetic risk, and DUSP23 expression showed that age > 60 (P< 0.001), poor & intermediate cytogenetic risk (P = 0.032), and high expression of DUSP23 (P = 0.008) were independent prognostic factors for worse OS (P < 0.05).

Then a Kaplan-Meier analysis was performed to examine the correlation between DUSP23 expression and AML patient prognosis. It was observed that poor prognosis was associated with high expression of DUSP23 in various subgroups, such as patients with age > 60 (P < 0.001; Figure 4A), BM blasts of > 20% (P = 0.009; Figure 4B), PB blasts <= 70% (P = 0.02; Figure 4C),

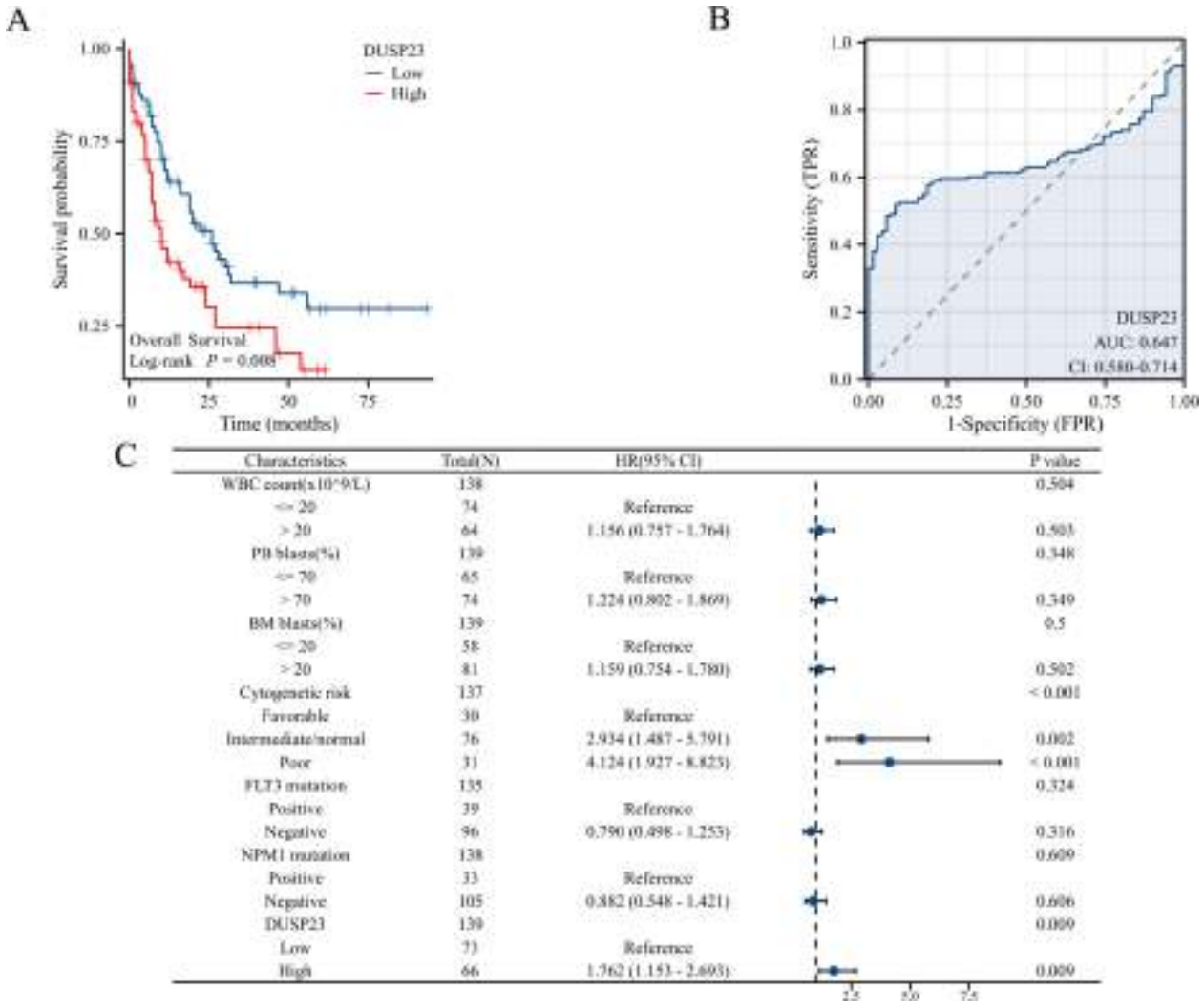

Figure 3 High expression of DUSP23 was associated with poor OS in AML patients. (**A**) Kaplan-Meier curves in all AML patients. (**B**) ROC analysis of DUSP23 in the prognosis of AML. (**C**) Forest plot showed that DUSP23 predicted poor prognosis in the subgroup of cytogenetic risk (Intermediate/normal andPoor) (HR=2.934, P= 0.002 and HR=4.124, P < 0.001), and DUSP23 high expression. (HR=1.762, P = 0.009).

FLT3 mutation positivity (P = 0.05), RAS mutation negativity (P = 0.023), IDH1 R132 mutation negativity (P = 0.007), R140 mutation negativity (P = 0.016), R172 mutation negativity (P = 0.011) (Figure 4D–H), and those with intermediate/normal and poor cytogenetic risk (P = 0.034; Figure 4I).

Prognostic Model of DUSP23 in AML

Moreover, based on multivariable logistic regression analysis, we established a nomogram that included the prediction model. The established nomogram was well-calibrated and demonstrated good discriminative power, with a C-index of 0.727 (0.700–0.754) for OS prediction (Figure 5A). Additionally, calibration curves were used to assess the clinical net benefit of our model, and the results showed high consistency between the predicted survival probability and actual OS proportions at 1, 3, or 5 years (Figure 5B).

Validation of Prognostic Gene Expression by Clinical Samples

To verify the expression level of DUSP23 between AML patients and healthy controls, qPT-PCR was detected in our cohort. The results showed that DUSP23 was significantly higher in AML patients than in healthy controls, which was

Table 3 Univariate and Multivariate Cox's Regression Analysis of Factors Associated with OS in AML

Characteristics	HR (95% CI) Univariate Analysis	P-value Univariate Analysis	HR (95% CI) Multivariate Analysis	P-value Multivariate Analysis
WBC count (x10^9/L)		0.504		
> 20	Reference			
<= 20	0.865 (0.567–1.321)	0.503		
PB blasts (%)		0.348		
> 70	Reference			
<= 70	0.817 (0.535–1.247)	0.349		
BM blasts (%)		0.500		
> 20	Reference			
<= 20	0.863 (0.562–1.326)	0.502		
Cytogenetic risk		< 0.001		
Favorable	Reference		Reference	
Poor&Intermediate/normal	3.184 (1.637–6.193)	< 0.001	2.130 (1.067–4.252)	0.032
Gender		0.912		
Male	Reference			
Female	0.976 (0.639–1.491)	0.912		
Age		< 0.001		
<= 60	Reference		Reference	
>60	0.301 (0.195–0.464)	< 0.001	0.305 (0.193–0.482)	< 0.001
Race		0.685		
White	Reference			
Black or African American&Asian	0.833 (0.337–2.060)	0.693		
FLT3 mutation		0.324		
Positive	Reference			
Negative	0.790 (0.498–1.253)	0.316		
IDH1 R132 mutation		0.210		
Positive	Reference			
Negative	1.706 (0.691–4.215)	0.247		
IDH1 R140 mutation		0.737		
Positive	Reference			
Negative	0.886 (0.443–1.773)	0.733		
IDH1 R172 mutation		0.591		
Positive	Reference			
Negative	1.644 (0.229–11.829)	0.621		
RAS mutation		0.355		
Positive	Reference			
Negative	1.558 (0.570–4.264)	0.388		
NPM1 mutation		0.609		
Positive	Reference			
Negative	0.882 (0.548–1.421)	0.606		
DUSP23		0.009		
High	Reference		Reference	
Low	0.568 (0.371–0.868)	0.009	0.547 (0.350–0.855)	0.008

consistent with the TCGA and GEO databases. (P= 0.0024; Figure 6A). Patients were then divided into high and low expression groups based on the cut-off value of DUSP23 mRNA expression. As expected, individuals with high DUSP23 expression showed poor survival compared to those with low expression (P = 0.0337; Figure 6B), as shown by the K-M statistic.

Notably, patients with higher DUSP23 expression had markedly higher WBC counts than those with lower DUSP23 expression (P = 0.026; Table 1). Univariate Cox proportional hazards regression was used to assess factors influencing

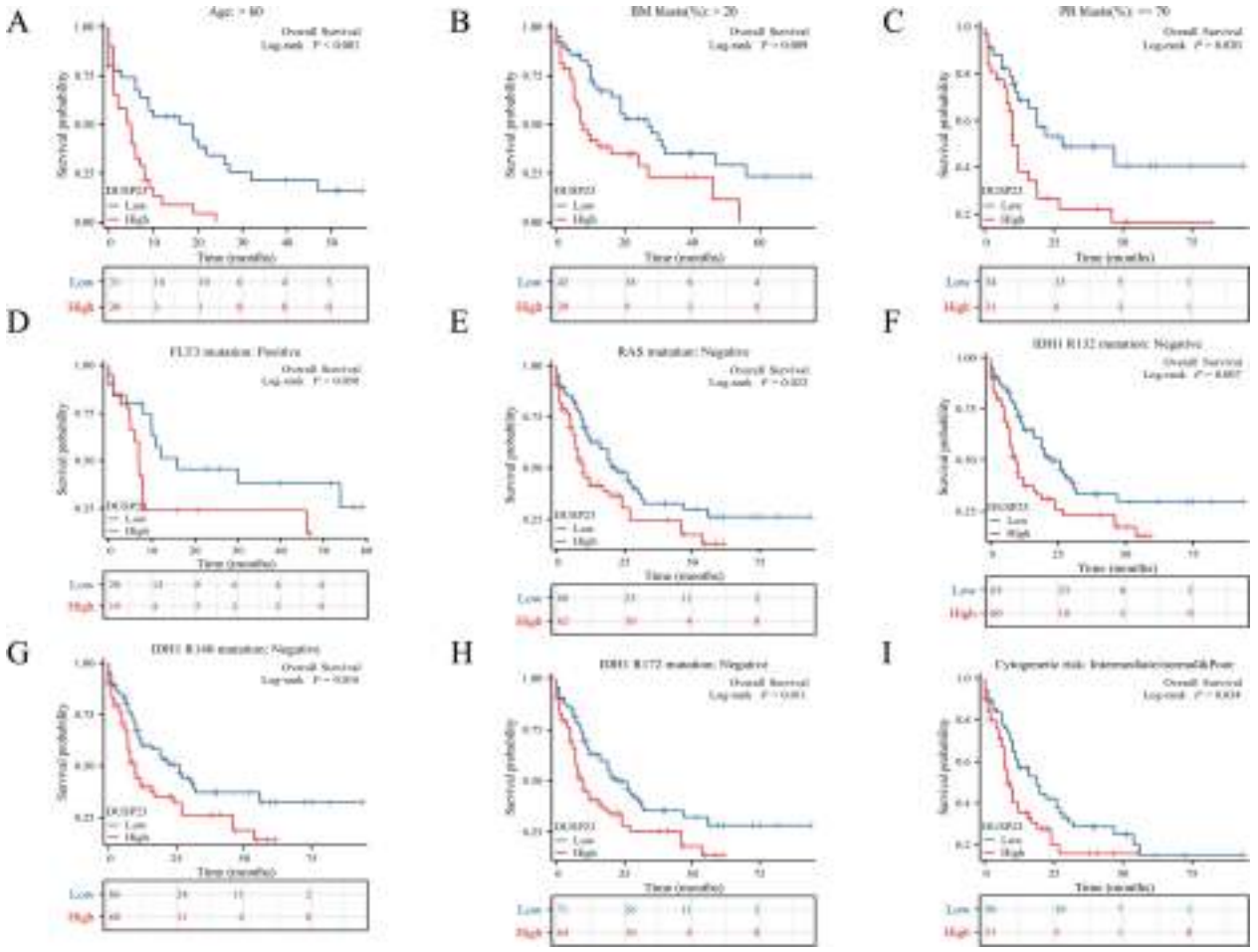

Figure 4 The impact of DUSP23 expression on survival of AML patients with specific subtypes. (**A**) Kaplan-Meier curves in AML patients with age ≥ 60. (**B**) Kaplan-Meier curves in AML patients with BM blasts > 20%. (**C**) Kaplan-Meier curves in AML patients with PB blasts ≤ 70%. (**D**–**H**) Kaplan-Meier curves in subgroups with FLT3 mutation-positive, RAS mutation-negative, IDH1 R132 mutation-negative, IDH1 R140 mutation-negative, R172 mutation-negative. (**I**) Kaplan-Meier curves in AML patients with cytogenetic risk intermediate/normal and poor.

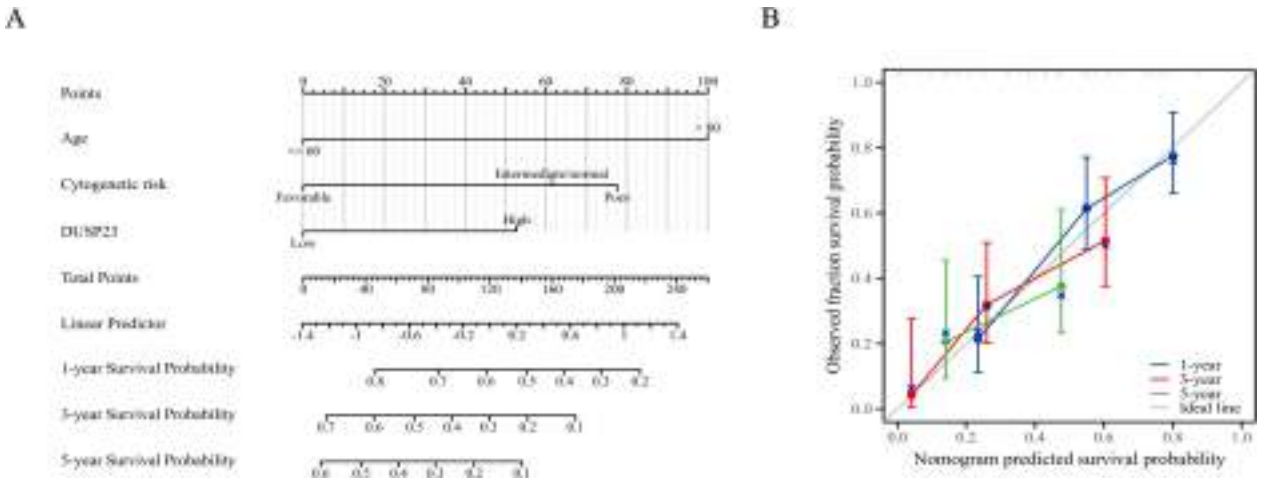

Figure 5 A prognostic predictive model of DUSP23 in AML. (**A**) Nomogram for predicting the probability of 1-, 3-, 5-year OS for AML. (**B**) Calibration plot of the nomogram for predicting the probability of OS at 1, 3, and 5 years.

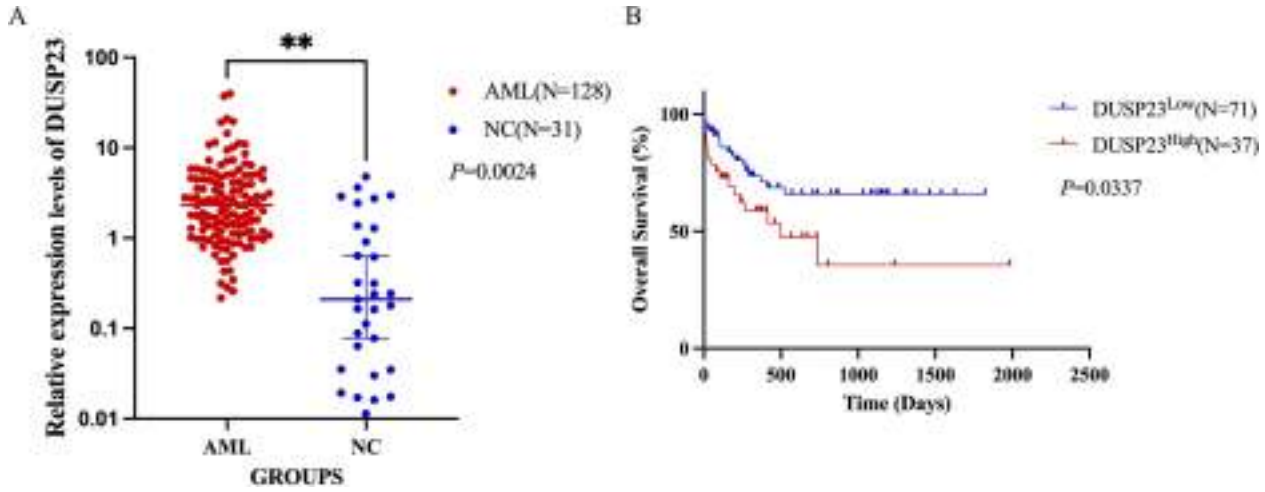

Figure 6 Quantitative real-time PCR results of DUSP23 expression. (**A**) qPT-PCR of DUSP23 (**B**) The overall survival analysis of DUSP23 related to AML patients (**P < 0.01).

OS. The results showed that high DUSP23 expression (P = 0.038), consolidation (P < 0.001) and age > 60 years (P = 0.013) were predictive factors for worse OS (Table 4). Subsequent multivariate Cox regression analysis including age, consolidation and DUSP23 expression showed that consolidation (P = 0.015) and high DUSP23 expression (P = 0.042) were independent prognostic factors for worse OS (P < 0.05). These findings highlight the potential of DUSP23 as a valuable prognostic indicator for survival in AML.

Biological Function Enrichment of the DUSP23 Gene in AML

Next, the gene expression profiles of high- and low-DUSP23 expression groups were utilized to further explore the biological function of DUSP23 in AML. There were 435 DEGs between high- and low-DUSP23 expression groups,

Table 4 Univariate and Multivariate Analysis of the Prognostic Significance of DUSP23 in AML

Characteristics	HR (95% CI) Univariate Analysis	P-value Univariate Analysis	HR (95% CI) Multivariate Analysis	P-value Multivariate Analysis
WBC count(x10^9/L)				
> 20	Reference			
<= 20	0.625 (0.311–1.255)	0.186		
Age				
<= 60	Reference		Reference	
>60	2.463 (1.208–5.019)	0.013	1.885 (0.860–4.129)	0.113
Consolidation				
HSCT	Reference		Reference	
Chemo	0.197 (0.076–0.514)	<0.001	0.284 (0.101–0.797)	0.015
DNMT3A mutation				
Positive	Reference			
Negative	0.149 (0.020–1.086)	0.06		
KIT mutation				
Positive	Reference			
Negative	0.169 (0.023–1.239)	0.08		
DUSP23				
High	Reference		Reference	
Low	2.027 (1.041–3.943)	0.038	2.060 (1.025–4.136)	0.042

including 141 up-regulated and 294 down-regulated genes (|log fold change (logFC)| > 1.5, P < 0.05) (Figure 1A). The top ten up- and down-regulated DEGs between the groups were presented in the heat map (Figure 1B).

To gain insights into the functional implications of the 435 DEGs concerning DUSP23 expression levels in AML, GO and KEGG functional enrichment analyses were performed. The target genes showed statistical significance in the GO terms related to biological processes such as embryonic organ development, regionalization, and morphogenesis, molecular functions including DNA-binding transcription activator activity, DNA-binding transcription activator activity/RNA polymerase II-specific, and carbohydrate binding, and cellular components such as collagen-containing extracellular matrix, endoplasmic reticulum lumen, and collagen trimer. The DEGs were found to be enriched in the KEGG pathways such as Staphylococcus aureus infection, complement, and coagulation cascades (Supplementary Figure 2).

Finally, GSEA enrichment analysis results show that DUSP23 may be related to multiple signaling pathways in hematological tumors, including BCR signaling, MAPK pathway, RAS regulation, PTEN regulation, HEDGEHOG signaling, WNT signaling, MYC pathway, and TP53 regulation (Figure 7A–H and Supplementary Table 1).

Identification of Hub Genes Associated with DUSP23 Expression

To perform a more in-depth analysis of the data, a PPI network related to DEGs was constructed and analyzed. The top 10 hub genes were identified using the maximal neighborhood component (MNC) and degree methods with the cytoHubba plug-in in Cytoscape (Figure 8A and B). It was discovered that nine hub genes were shared between the two gene lists, namely ATP5F1D, NDUFB7, POLR2L, MRPS12, MRPL2, UBA52, IMP3, UQCRQ, and MRPL4. Significant associations were found between DUSP23 and ATP5F1D (P < 0.001, correlation coefficient: 0.702), NDUFB7 (P < 0.001, correlation coefficient: 0.731), POLR2L (P < 0.001, correlation coefficient: 0.702), MRPS12 (P< 0.001, correlation coefficient: 0.780), MRPL2 (P < 0.001, correlation coefficient: 0.498), UBA52 (P < 0.001, correlation coefficient: 0.637), IMP3 (P < 0.001, correlation coefficient: 0.512), UQCRQ (P < 0.001, correlation coefficient: 0.591), and MRPL4 (P < 0.001, correlation coefficient: 0.708) (Figure 8C). Finally, the impact of hub gene levels on patient prognosis was examined, and it was discovered that IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D are closely linked to unfavorable clinical outcomes in individuals with AML (Figure 8D–H).

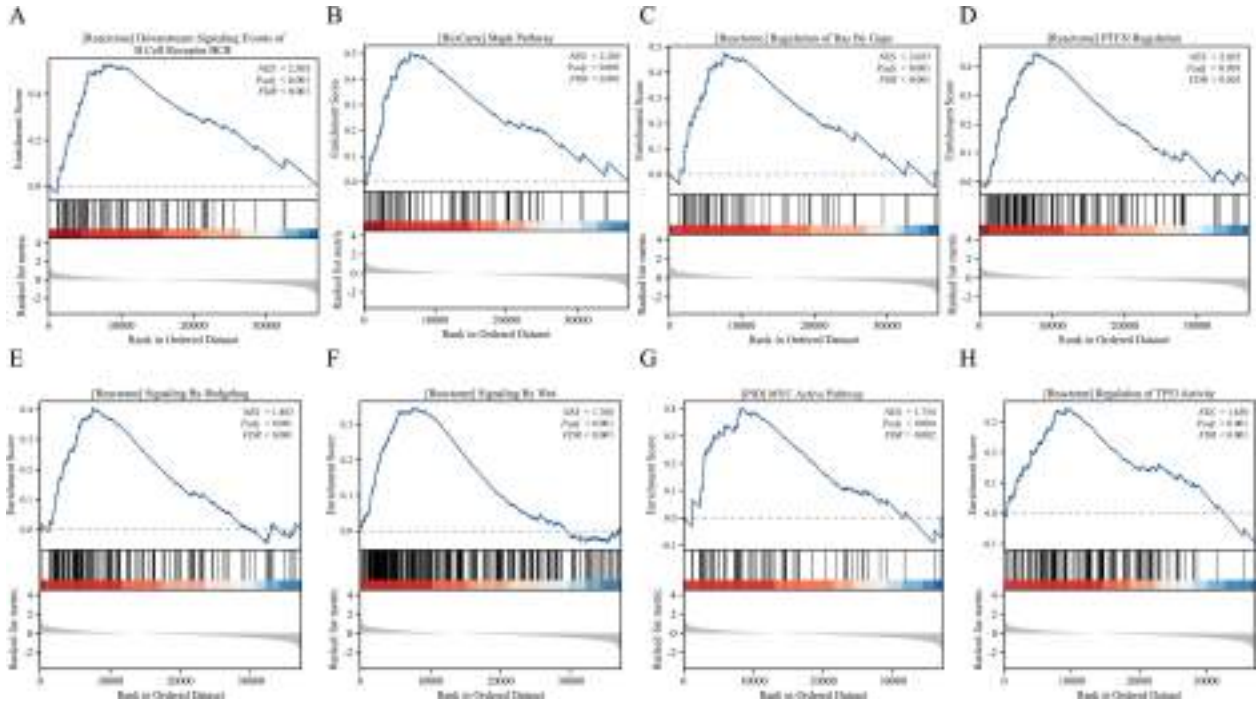

Figure 7 Enrichment plots from the gene set enrichment analysis (GSEA). (**A–H**) ES, enrichment score; NES, normalized ES; ADJ P- val, adjusted P-value.

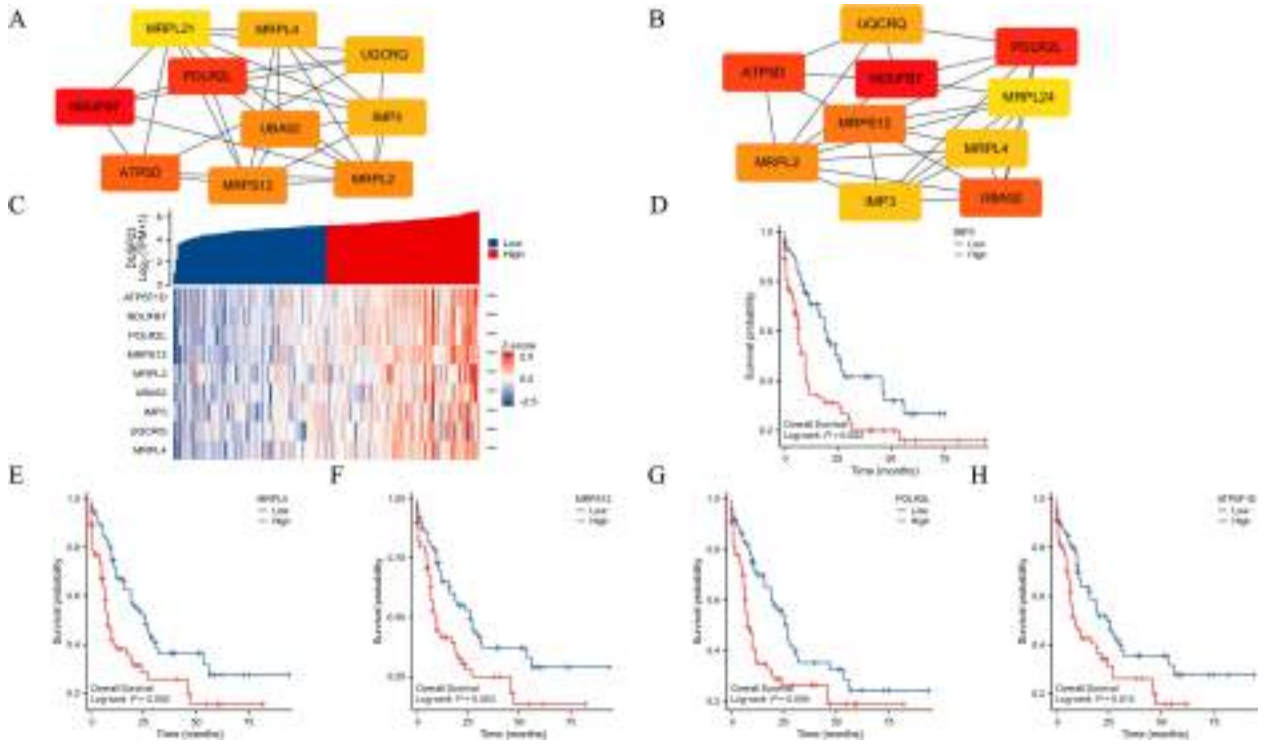

Figure 8 PPI network construction and clinical significance of hub genes. (**A** and **B**) The top 10 hub genes were selected based on (**A**) MNC and (**B**) degree. (**C**) The association of DUSP23 with nine hub genes (ATP5F1D, NDUFB7, POLR2L, MRPS12, MRPL2, UBA52, IMP3, UQCRQ, and MRPL4) (***P < 0.001). (**D**–**H**) The difference in OS between patients with high and low IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D expression levels is shown by Kaplan-Meier curves.

Immune Infiltration Analysis in AML

In the AML microenvironment, a positive correlation was found between the expression level of DUSP23 and the number of various immune cells, including NK CD56 bright cells, NK cells, TFH, TReg, Eosinophils, NK CD56dim cells, aDC, CD8 T cells, Mast cells, Cytotoxic cells, Neutrophils, iDC, Macrophages, Th17 cells, Th1 cells, and DC, as revealed by the correlation analysis of immune cell infiltration quantified by SSGSEA. Particularly, a significant association with DUSP23 was exhibited by NK CD56 (bright) cells, indicating that DUSP23 may serve as a significant immune marker in AML (Figure 9).

Discussion

The present study showed that the mRNA expression level of DUSP23 was significantly higher in AML patients than healthy controls, and DUSP23 high expression was an independent adverse prognostic factor for OS. In addition, The MAPK signaling pathways may be stimulated by increased DUSP23 expression levels, which may be important for the development of AML.

By exploring the association between DUSP23 gene expression levels and the main clinical features in TCGA-LAML cohorts, it was revealed that DUSP23 was significantly upregulated in AML patients. Notably, high levels of DUSP23 were associated with higher PB blast levels, intermediate/normal & poor cytogenetic risk, FLT3 mutation, and poor prognosis. FLT3 mutations are detected in approximately 30% of AML patients and are associated with unfavorable outcomes.[25] Therefore, we speculate that abnormally high expression of DUSP23 may have a negative impact on the survival of AML patients.

qPT-PCR was further performed to validate the differential expression of DUSP23 in AML and healthy controls. The results revealed a significant upregulation of DUSP23 in AML patients, and poor survival was observed in those with high DUSP23 expression. Using multivariate Cox regression analysis, we identified high DUSP23 expression as an independent prognostic factor along with consolidation. Therefore, DUSP23 can be considered as a novel adverse prognostic factor in AML patients.

Furthermore, GSEA showed enrichment of MAPK, RAS, PTEN, WNT, MYC, and TP53 pathways. The MAPKs are a family of serine-threonine kinases that play a crucial role in regulating the proliferation and differentiation of both normal and malignant

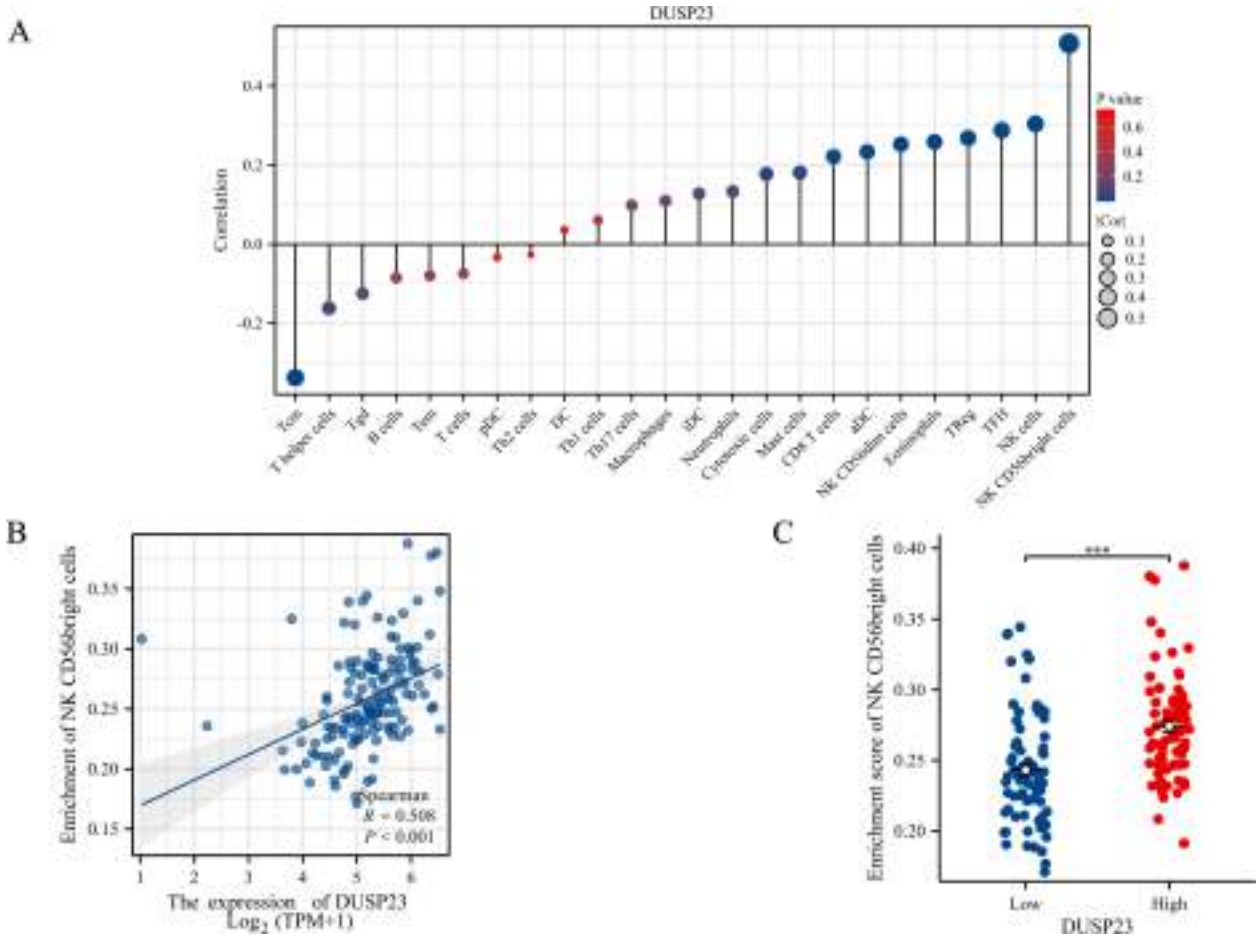

Figure 9 Correlation analysis between the level of DUSP23 expression and immune cell infiltration. (**A**) The relative contents of 24 kinds of immune cells in AML. (**B**) Correlation between the relative enrichment score of NK CD56(bright) cells and the expression level (TPM) of DUSP23. (**C**) Infiltration of NK CD56(bright) cells between low- and high-DUSP23 expressed (***P < 0.001).

hematopoietic cells.[26,27] MAPK contributes to the development of various cancers, such as colorectal, ovarian, and breast cancers, as well as AML.[28–30] The molecular mechanisms responsible for maintaining a balance between activated RAS-GTP and inactive RAS-GDP are complex and involve multiple factors, including protein kinases, scaffolding proteins, phosphatases, GAPs, and GEFs.[31] Mutations in genes encoding these components of the intricate network have been identified in inherited genetic syndromes called RASopathies, as well as in sporadic cancers, including AML. Notably, these mutations occur with a high frequency in juvenile myelomonocytic leukemia (JMML).[32] PTEN is an important tumor suppressor gene frequently inactivated in human cancer. Its inactivation has been shown to have crucial roles in the generation of leukemia stem cells.[33] In AML, mutations in hematopoietic stem/progenitor cells result in upregulated Wnt signaling through various mechanisms. Wnt signaling is crucial for maintaining leukemic stem cells.[34] Although the impact of MYC protein expression in AML is not well understood, dysregulation of MYC has been implicated in AML. Most AML cases exhibit high levels of MYC protein, which is correlated with a worse prognosis.[35] TP53, a frequently mutated tumor suppressor gene in human cancer, remains intact in most AML cases, suggesting potential for harnessing its physiological tumor-suppressive roles. Therefore, pharmacological activators of the TP53 pathway may offer clinical benefits in AML. Conversely, despite the lower frequency of TP53 mutations in AML compared to other cancers, TP53 mutations are associated with chemoresistance and a high risk of relapse.[36] Thus, we speculated that the pathological mechanism of DUSP23 may be related to these signaling pathways.

In addition, through a series of rigorous screens, five hub genes that could accurately predict the prognosis of AML were found. IMP-3 binds to and promotes the translation of insulin-like growth factor II (IGF-II) mRNA among its many mRNA targets.[37] Reports suggest that reducing IMP-3 levels through siRNA-mediated knockdown decreases the proliferation of human

K562 chronic myeloid leukemia cells by reducing IGF-II biosynthesis.[38] Similarly, inhibiting IMP-3 in AML may potentially reduce cell proliferation. MRPL4 has been identified as a high-risk factor for prostate cancer (PC) and a potential diagnostic biomarker.[39] Additionally, MRPS12 has been implicated in ovarian cancer.[40] Several reports indicate that POLR2L plays a significant role in PC, glioblastoma (GBM), and HBV-related hepatocellular carcinoma (HCC).[41–43] Targeted specific demethylation of ATP5D m1A using the dm1ACRISPR system significantly enhances the expression of ATP5D and glycolysis in cancer cells. In vivo, data confirm the involvement of m1A/ATP5D in tumor growth and cancer progression.[44] However, further experiments are required to verify the mechanism of action among these genes.

In the analysis of immune cell infiltration, higher levels of CD56 (bright) NK cells were found to be associated with high expression of DUSP23. CD56 (bright) NK cells, which are considered to be efficient cytokine producers with immunoregulatory properties, can also become cytotoxic upon appropriate activation and have been shown to play a role in various disease states, including cancer, autoimmunity, neuroinflammation, and infection.[45,46] In this study, a positive correlation was observed between the infiltration of CD56 (bright) NK cells and the expression of DUSP23. Kaplan-Meier survival analysis indicated that poor prognosis in AML patients was associated with high expression of DUSP23. However, the relationship between CD56 (bright) NK cells and AML has not been fully clarified. Therefore, further exploration is required to elucidate the relationship between DUSP23 and CD56 (bright) cells and the potential involvement of DUSP23 and CD56 (bright) NK cells in immune escape in AML.

Our study revealed DUSP23 expression is higher in AML patients than healthy controls and high DUSP23 expression indicate a poor prognosis. Furthermore, we found that the potential biological function of DUSP23 in AML may be mediated by activation of MAPK signalling pathways. However, this was a retrospective follow-up study. To define the role of DUSP23 in AML more precisely, further research with a larger sample size of AML is needed. In addition, the mechanism of DUSP23 in AML needs to be confirmed in the future.

Conclusion

Our findings have revealed that an unfavorable impact on the OS of AML patients was observed in cases where the gene DUSP23 was highly expressed. In addition, high levels of DUSP23 were identified as an independent predictor of poor prognosis in AML patients.

Abbreviations

DUSP23, Dual-specificity Phosphatase 23; AML, acute myeloid leukemia; TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; DEGs, differentially expressed genes; OS, overall survival; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; GSEA, gene set enrichment analysis; PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes; ATRA, all-trans retinoic acid; ATO, arsenic trioxide; C-index, concordance index; AUC, area under the curve; WBC, white blood cell; MNC, the maximal neighborhood component; qRT-PCR, Quantitative real-time PCR; IGF-II, insulin-like growth factor II; JMML, juvenile myelomonocytic leukemia; PC, prostate cancer; GBM, Glioblastoma; HCC, HBV-related hepatocellular carcinoma.

Data Sharing Statement

The databases generated and/or analysed during the current study are available in the GEO, TCGA, and GETx repository, https://portal.gdc.cancer.gov; https://xenabrowser.net/datapages/; https://www.ncbi.nlm.nih.gov/geo/.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki. This study was conducted under the approval of the Ethics Committee of the Affiliated People's Hospital of Ningbo University. All methods were carried out in accordance with relevant guidelines and regulations. (Approval NO.: the Affiliated People's Hospital of Ningbo University Ethics review 2023-research-107).

Author Contributions

Each author has carefully reviewed and endorsed the final manuscript. They have collectively contributed significantly to various aspects of the research, including conception, study design, execution, data acquisition, analysis, and interpretation. All authors actively participated in drafting, revising, and critically reviewing the article. They have provided final approval for the version submitted for publication, unanimously agreed on the selected journal, and committed to being accountable for all facets of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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ORIGINAL RESEARCH

Assessing Nutritional Anemia Among University Students in Jazan, Saudi Arabia: A Public Health Perspective

Waleed Hakami[1], Gasim Dobie[1], Khadija A Alneami[1], Misk Shaabi[1], Khaled Essawi[1], Muhammad Saboor[2], Aymen M Madkhali[1], Mohammed H Nahari[3], Hassan H Almasoudi[3], Mohammad S Akhter[1], Fasial H Hakami[4], Fatimah A Zarbatan[5], Ali Hakamy[6], Rama M Chandika[7], Ali A Fageehi[1], Abdullah A Mobarki[1], Hassan A Hamali[1]

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Background: Nutritional anemia is a significant public health concern worldwide, particularly affecting young adults and children in Saudi Arabia, where inadequate nutrition is considered a primary contributing factor. This study aims to (i) examine the levels of serum iron, folate, and vitamin B_{12} in young adult students, with a focus on identifying any deficiencies and their association with anemia; (ii) explore the prevalence of mixed-deficiency anemia resulting from deficiencies in serum iron, folate, and vitamin B_{12} (iii) explore how sociodemographic characteristics and dietary habits influence serum iron, folate, and vitamin B_{12} levels.

Materials and Methods: This cross-sectional study encompassed 158 young adult students at Jazan University, Saudi Arabia. Blood samples were collected following a comprehensive questionnaire addressing sociodemographic and health characteristics. These samples were analyzed for complete blood count, serum iron, folate, and vitamin B_{12} levels.

Results: The findings of this study revealed a significant decrease in serum iron levels, with 70.6% of males and 88% in females exhibiting reduced level. Additionally, low levels of folate were observed in 4% of the study population, while deficiency in vitamin B_{12} was found in 2.2% of the study population. However, the simultaneous presence of low serum iron levels along with deficiencies in folate or vitamin B_{12} was not observed in the study participants.

Conclusion: The study indicates that there is a high incidence of low serum iron and ferritin levels among university students in Saudi Arabia, which poses a considerable public health concern. Conversely, the prevalence of folate and vitamin B_{12} deficiencies among the students was comparatively low, and notably, there were no cases where these deficiencies were observed alongside iron deficiency.

Keywords: anemia, folate, vitamin B_{12}, serum iron, iron deficiency

Introduction

Nutritional anemia is a prevalent health burden that affects individuals across all ages, with higher vulnerability observed among young children, women, and older adults.[1,2] Anemia is characterized by a low number of healthy circulating red blood cells (RBCs) or insufficient amount of hemoglobin (Hb) to carry oxygen.[2,3] Micronutrients, such as vitamins (A, B, C) and minerals (iron, zinc, iodine, calcium), play diverse and essential roles in the body, including proper growth, development, and blood formation.[4] Inadequate intake of these micronutrients, particularly iron, folate, and vitamin B_{12}, is considered the primary contributing factor to anemia.[5] A recent study conducted in 2021 estimated that anemia influences approximately

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1.92 billion individuals worldwide, with prevalence rate of 24.3%. Iron deficiency (ID) is the most common cause of nutritional anemia, followed by deficiencies in folate and vitamin B_{12}.[2,6,7] Developing nations experience the greatest impact, characterized by a prevalence exceeding 60% and estimated rates of 47.4%, 41.8%, and 30.2% among young children, pregnant women, and women of reproductive age, respectively.[6,8]

A nationwide study conducted in four regions of Saudi Arabia unveiled a significant prevalence of anemia, with rates ranging from 16.5% to 41.3% among children, 7.2% to 16.5% among adult males, and 10.8% to 23.5% among adult females.[9] Remarkably, a recent study documented that 67% of young female students encountered either iron deficiency anemia (IDA) or ID without anemia.[10] Moreover, a cohort study conducted on 1312 adults enrolled in a premarital screening program reported an incidence rate of 7.23% for acquired anemia in Jazan region.[11] This observation further underscores the burden of nutritional anemia in the Jazan region.

Micronutrients, specifically vitamin B_{12} and folate, play essential roles in DNA synthesis, cell division, and the production of RBCs.[12] Insufficient levels of these micronutrients can lead to impaired DNA synthesis, which in turn can contribute to the development of macrocytic anemia, a condition that is relatively less frequently reported in Saudi Arabia. A retrospective study conducted in Makkah hospitals investigated the occurrence of macrocytic anemia in relation to folate and vitamin B_{12} deficiencies among male and female patients aged over 15 years. The study found that deficiencies in folate and vitamin B_{12} were linked with a 2% incidence of macrocytic anemia in this population. Additionally, another study carried out in Asir Central Hospital in Abha City reported a 1.6% incidence of only folate deficiency.[13] Moreover, there is a significant occurrence of subclinical inadequacy and deficiency in vitamin B_{12} within specific societies, with estimates suggesting rates as high as 60%.[14–18] In Saudi Arabia, especially in Jazan region, data is remain limited regarding nutritional anemia. Hence, further studies are required to determine the prevalence of nutritional anemia caused by deficiencies in serum iron, folate, and vitamin B_{12}. Furthermore, it is crucial for these investigations to explore additional variables including socio-demographic factors and dietary patterns to obtain a more comprehensive understanding of the situation. Consequently, the objectives of this study encompassed three aspects: (i) determining the prevalence of nutritional anemia resulting from deficiencies in serum iron, folate, and vitamin B_{12} among young male and female students, (ii) investigating the co-occurrence of low serum iron levels with deficiencies in folate and/or vitamin B_{12}, and (iii) assessing the relationship between socio-demographic characteristics, dietary intakes, and circulating levels of iron, folate, and vitamin B_{12}.

Materials and Methods

Study Design

A cross-sectional study was undertaken on a sample consisting of 158 young adult students (68 males and 90 females), ranging in age from 18 to 25 years, who were enrolled at the Faculty of Applied Medical Sciences, Jazan University. The participants included in the study exhibited no apparent signs of illness. The survey questionnaire was employed to collect sociodemographic information, including age, weight, height, marital status, and personal history of any chronic diseases. The selection of participants was randomized, with exclusion criteria applied for individuals with known chronic conditions and pregnant females.

Ethical Consideration

The present study underwent a thorough review and received approval from the Standing Scientific Research Ethics Committee of Jazan University, with the reference number REC-44/06/478. The study was conducted in strict adherence to the principles outlined in the Declaration of Helsinki.

Sample Size and Collection

The sample size was determined based on a previously established method.[10] Venous blood samples were obtained from all participants using ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes for complete blood count (CBC) analysis, and plain tubes were used for serum iron, serum vitamin B_{12}, and folate analyses.

Complete Blood Count

Sysmex XN–1000 (Japan) was used for complete blood count analysis. The CBC report included red blood cells (RBC, $x10^{12}$/L), hemoglobin (Hb, g/dL), hematocrit (Hct, %), and RBC indices, including mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), red cell distribution width (RDW, %), white blood cell count (WBC, $x10^9$/L), differential leukocyte count, and platelet count (10^9/L).

Biochemical Analysis

The levels of iron, ferritin, folate, and vitamin B_{12}, were determined by analyzing serum samples. The measurements of ferritin, folate, and Vitamin B_{12} were performed using a Maglumi 600 Chemiluminescence Immunoassay system (China), while iron levels were determined using a HumaStar 200 chemistry analyzer (Germany).

Reference Values

Anemia was defined as hemoglobin (Hb) <12.0 g/dL in females and <13.0 g/dL in males.[19] MCV was used to classify anemia morphologically. MCV greater than 100 fL, along with low Hb levels, indicated macrocytic anemia, while MCV less than 80 fL, coupled with low Hblevels, indicated microcytic anemia. The local reference values for serum iron were 80–180 µg/dL in males and 60–160 µg/dL in females.[10] Ferritin levels ranged from 24 to 336 µg/L in male and 11 to 307 µg/L in female.[20] Folate levels ranged from 3.1 to 17.5 ng/mL and vitamin B_{12} levels ranged from 211 to 950 pg/mL.[21] Values below the normal reference intervals were considered low (deficiency), while values above the upper limit were considered high.

Statistical Analysis

Data from the current study were analyzed using the GraphPad Prism software (version 8.0; San Diego, CA, USA). Unless otherwise stated, the results are presented as mean ± standard deviation (SD). CBC parameters, folate, vitamin B_{12}, serum iron and ferritin values were analyzed using independent unpaired Student's *t*-test for normally distributed and Mann–Whitney for non-normally distributed. The chi-square test was used for demographic data analysis and Pearson correlation test was used for correlation study. P-values less than 0.05 were considered statistically significant.

Results

Sociodemographic and Health Characteristics

The sociodemographic and health characteristics of the study participants (68 males and 90 females) are presented in Table 1. The participants exclusively consisted of Saudi students enrolled at the College of Applied Medical Sciences, Jazan University. Although the mean age of the male group was comparable to the female group ($p > 0.05$), the weight (kg), height (cm), and BMI were significantly higher in the male group as compared to the female group ($p < 0.001$) (Table 1). All male participants were single, with 75% being non-smokers, whereas 77.8% of the female participants were single, and 95.6% were non-smokers. The nutritional habits displayed some variations between the male and female participants (Table 1). There was a significant difference between male and female in nutritional habits ($p < 0.05$) expect for daily milk consumption and participants following a diet ($p > 0.05$) (Table 1).

Comparative Analysis of Vitamin B_{12}, Folate, and Serum Iron Levels in Males and Females

The study compared the serum levels of iron, folate, and vitamin B_{12} between males and females (Table 2). Serum iron and ferritin levels were significantly lower in females compared to males ($p < 0.0001$). However, the levels of folate were comparable between the male and female ($p > 0.05$), while vitamin B_{12} levels were significantly higher in females than in males ($p < 0.01$).

Table 1 Socio-Demographic, Health Characteristics and Nutritional Habits of the Study Participants (Male = 68 and Female = 90).

Variables		Male (n = 68)	Female (n = 90)	P value
		n (%)	n (%)	
Age/year		22.4±2.4	21.9±1.9	0.097
Weight/kg		71.7±23.2	54.2±17.2	<0.0001
Height/cm		171.0±5.0	153.7±6.2	<0.0001
BMI		25.5±5.3	22.6±4.9	0.0005
Marital status	Single	68 (100)	70 (77.8)	<0.0001
	Married	0 (0)	20 (22.2)	
Smoking	Yes	17 (25)	4 (4.4)	<0.0001
	No	51 (75)	86 (95.6)	
Vitamin supplementation	Yes	3 (4.4)	19 (21.1)	0.009
	No	65 (95.6)	71 (78.9)	
Exercise (3 /week)	Yes	28 (41.2)	9 (10)	<0.0001
	No	40 (58.8)	81 (90)	
Regular meals (3 meals/day)	Yes	28 (41.2)	21 (23)	<0.0001
	No	40 (58.8)	69 (77)	
Fast food (5 time/week)	Yes	57 (83.8)	86 (95.6)	0.046
	No	11 (16.2)	4 (4.4)	
On diet	Yes	3 (4.4)	8 (8.9)	0.292
	No	65 (95.6)	82 (91.1)	
Consumption of red meat	Yes	58 (85.3)	28 (31.1)	<0.0001
	No	10 (14.7)	62 (68.9)	
Daily drink milk	Yes	48 (70.6)	61 (67.8)	0.536
	No	20 (29.4)	29 (32.2)	
Fruit consumption	Yes	34 (50)	17 (18.9)	<0.0001
	No	34 (50)	73 (81.1)	
Vegetable consumption	Yes	39 (57.3)	19 (21.1)	<0.0001
	No	29 (42.7)	71 (78.9)	

Gender-Based Distribution of Folate Levels in the Study Population

Serum Iron and Ferritin Levels

Among males, 70.6% and 8.8% exhibited significantly low levels of serum iron and ferritin, respectively. In the female population, 88% and 21% had significantly low levels of serum iron and ferritin, respectively (Table 3 and Figure 1).

Folate Levels

In the male population, all study participants had normal folate levels, while in the female population, 97% (n = 88) had normal folate levels, and 2% (n = 2) had low levels (Table 3 and Figure 1).

Table 2 Comparison of Mean Levels of Folate, Vitamin B_{12} and Serum Iron Between Young Adult Males (n = 68) and Young Adult Females (n = 90).

Parameters	Male (n = 68)	Female (n = 90)	P value
Folate (ng/mL)	10.7±4.6	9.7±5.1	0.2051
Vitamin B_{12} (pg/mL)	555±347	692±243	0.0040
Serum iron (µg/dL)	65±29	40±34	<0.0001
Serum ferritin (µg/L)	71.3±53	26±23	<0.0001

Table 3 Levels of Folate, Vitamin B_{12}, Serum Iron and Serum Ferritin in Female and Male Population According to the Normal Range.

Parameters	Male (n=68)			% Prevalence of deficiency	Female (n=90)			% Prevalence of deficiency
	Low	Normal	P value		Low	Normal	P value	
Folate (ng/mL)	0 (n = 0)	10.7±4.1 (n = 68)	NA	0	1.5±0.6 (n = 2)	4.9±1.5 (n = 88)	NA	3%
Vitamin B_{12} (pg/mL)	208±2 (n = 3)	570±348 (n = 65)	<0.0001	4%	0 (n = 0)	224±114 (n = 90)	NA	0
Serum iron (µg/dL)	50.1±14.8 (n = 48)	102.1±14.4 (n = 20)	<0.0001	70.6%	28.2±18.9 (n = 79)	99.1±36.5 (n = 11)	<0.0001	88%
Serum ferritin (µg/L)	12.3±4 (n = 6)	78.5±52 (n = 62)	<0.0001	8.8%	7.3±2.6 (n = 19)	58.1±40.4 (n = 71)	<0.0001	21%

Vitamin B_{12} Levels

In the male population, 96% of the study participants had normal levels of vitamin B_{12}, while 4% had low levels. In contrast, all female in the study population had normal levels of vitamin B_{12} (Table 3 and Figure 1).

Co-Existence of Low Serum Iron Levels with Folate and/or Vitamin B_{12}

The data report no co-existence of low serum iron levels with folate and/or vitamin B_{12} (data not shown).

Anemia Among Study Participants

The CBC data for both male and female participants are presented in Table 4. The female population was divided into two groups based on mean Hb concentration: anemic (Hb < 12.0 g/dL) and normal (Hb > 12.0 g/dL), following WHO criteria for anemia.[10] 51.1% of the female participants (n = 46) were classified as anemic, with a mean Hb concentration of 10.4 ± 1.3 g/dL, while the remaining 49.9% had normal Hb levels, with a mean Hb concentration of 13.1 ± 0.8g/dL ($p < 0.01$). Notably, CBC parameters in anemic females were significantly lower than those in normal females ($p < 0.01$), except for the RDW (Table 4). Both anemic and normal females exhibited significantly low mean levels of serum iron and ferritin (Table 4; $p > 0.05$). The MCV data indicated the presence of microcytic anemia in the female group (Table 4). Surprisingly, anemic females had significantly higher levels of vitamin B_{12} than normal females (722 ± 263 vs 660 ± 239; $p < 0.05$), while no significant difference in folate levels were observed (9.6 ± 5.3 vs 9.8 ± 4.7; $p > 0.05$) (Table 4).

In contrast, all male participants were non anemic (Table 4). Among the male population, 67.6% (n = 46) exhibited low mean levels of serum iron, while 32.4% (n = 22) had normal levels (50 ± 15 vs 101 ± 18; $p < 0.0001$) (Supplementary Table 1). The CBC parameters were comparable between the male population with low serum iron and those with normal levels, except for the RDW, MCH, and MCHC, which were significantly higher in the iron deficient male population (Supplementary Table 1). Although the study indicated a low incidence rate of low folate and vitamin B12 levels, macrocytic anemia was not observed.

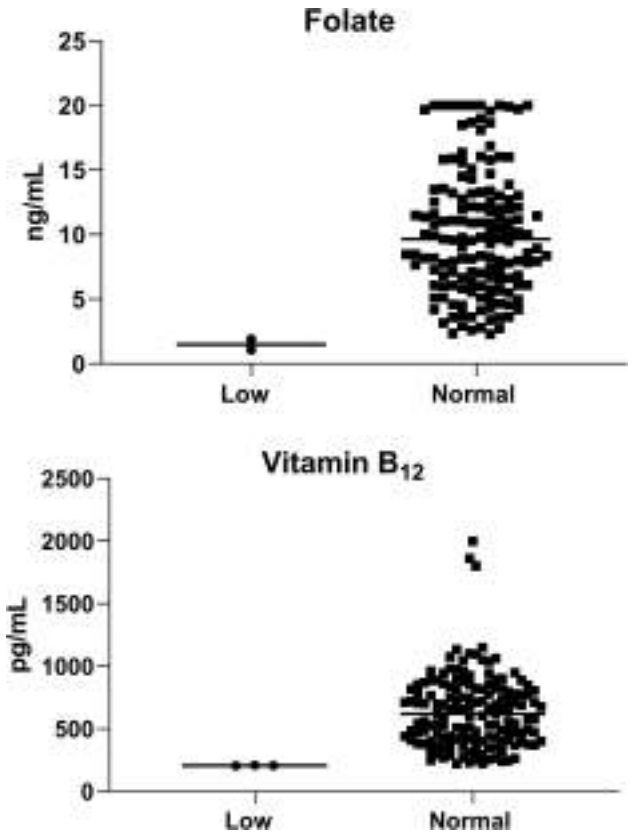

Figure 1 Levels of folate, and vitamin B_{12} among the study population according to the normal range (total = 158; male = 68 and female = 90).

Correlation Between Study Parameters (Hemoglobin, Vitamin B_{12}, Folate, Serum Iron, Ferritin, Sociodemographic Characteristics, and Nutritional Habits)

In the male population, a significant positive correlation was observed between vitamin B_{12} and folate levels ($R = 0.25$, $p < 0.044$). However, no significant correlations were found between vitamin B_{12} and serum iron, ferritin, or hemoglobin levels. Folate, on the other hand, showed a significant positive correlation with serum iron. Additionally, a negative correlation was observed between folic acid and age in the male population only (Supplementary Table 2).

In the female population, a significant positive correlation was found between vitamin B_{12} and folate levels ($R = 0.22$, $p < 0.044$). Serum iron, on the other hand, showed a significant negative correlation with hemoglobin levels and low red meat consumption (Supplementary Table 2).

Discussion

The current study reported significantly low serum iron levels among young adult males (70.6%) and female (88%), while folate and vitamin B_{12} deficiencies were only found in 2.2% and 4% of participants, respectively. Low serum iron and ferritin levels were significantly associated with low Hb levels ($p < 0.05$) and a high incidence rate of microcytosis (51.1%) in female population, contributing to the development of ID and IDA, which was not observed in the male cohort. These findings are consistent with previous studies conducted in the Jazan region[10] and in other parts of Saudi Arabia.[9–11,22–34] The high prevalence of ID and IDA among young and childbearing females in the Saudi community has been attributed to nutritional habits, including skipping breakfast and low consumption of red meat.[10,22,26,35,36] In the current study, the female population showed strong association between low serum iron and low red-meat consumption. Indeed, low consumption of red meat (less than 2 times per week) had been found to be associated with the development of IDA among female students in Saudi Arabia.[32] Other factor including menstrual cycle in females has been linked to

Table 4 Red Blood Cell Parameters, Serum Iron, Vitamin B_{12} and Folic Acid of Male and Female Populations

Parameters	Male (n = 68)	Female (90)		
	Normal (Hb > 13.0g/dL) (n=68)	Normal (Hb>12.0g/dL) (n=44)	Anemic (Hb<12.0g/dL) (n=46)	P value
RBCs ($x10^{12}$/L)	5.4±0.4	4.8± 0.5	4.6±0.5	0.0055
Hgb (g/dL)	15.3± 1.0	13.1 ± 0.8	10.4±1.3	<0.0001
Hct (%)	45.9± 3.5	37.1± 2.8	31.3± 4.2	<0.0001
MCV (fL)	85.0±4.5	77.3±5.6	68.8±9.0	<0.0001
MCH (pg)	28.5± 2.2	27.3±2.3	22.9± 3.5	<0.0001
MCHC (g/dL)	33.3±1.4	35.3±1.4	33.1±2.3	<0.0001
RDW (%)	17.3± 3.4	21.1±2.8	21±3.3	0.7573
Folic acid (ng/mL)	10.9±4.8	9.6±5.3	9.8±4.7	0.4759
Vitamin B_{12} (pg/mL)	810± 538	660±239	722±263	<0.0001
Serum iron (µg/dL)	65±29	47±39	36±27	0.1220
Serum ferritin (µg/L)	71.3±53	48±39	18± 19	<0.0001

Abbreviations: RBC, red cell count; Hb, hemoglobin; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW; red cell distribution width.

the development of IDA.[22,36] Furthermore, previous studies has attributed the high incidence of low iron levels and ID to irregular meals,[10,26,35,36] which is very high among males and females in the current study.

To the best of our knowledge, this study represents the first investigation into folate and vitamin B_{12} levels among young male and female students in Jazan region. The current study revealed noteworthy gender differences. Folate deficiency was more prevalent in females (2.2%) compared to males (0%), while vitamin B_{12} deficiency was more frequent in males (4%) than in females (0%). The findings regarding folate levels were in line with a study conducted in Abha city (2.8%). However, there was a disparity in the findings concerning vitamin B_{12} levels, as the Abha city study reported a considerably higher prevalence of 17.2% for vitamin B_{12} deficiency, which did not align with our results.[13] In our study, no case of vitamin B_{12} deficiency was reported in the female population. This finding contradicts a study conducted at King Saud University in Riyadh, where 6% of childbearing females were found to have vitamin B_{12} deficiency.

Comparatively, the prevalence of folate and vitamin B_{12} deficiencies in our study was lower than in some other Middle Eastern countries.[37] For instance, in Iran, the prevalence of folate and vitamin B_{12} deficiencies was reported to be 16.8% and 6.1%, respectively.[37] Globally, the rate of folate and vitamin B_{12} deficiencies varies widely, ranging from 2.5 to >20% depending on factors such as age, sex, and ethnic background.[13–16,18,37,38] Therefore, the relatively low incidence of folate and vitamin B_{12} deficiencies in Saudi Arabia may be attributed to the high consumption of animal products and fortified wheat flour, which are rich in folate and vitamin B_{12}.[39]

In addition to dietary factors, several other reasons contribute for folate and vitamin B_{12} deficiencies and the development of anemia in young women. These include repeated pregnancies, adherence to strict vegetarian diets, the use of certain medications, and various clinical conditions.[40] Low folate levels has been linked to many pathological conditions such as cancer and cardiovascular disorders.[41] Conversely, sufficient folate intake is associated with health benefits, including a reduced risk of cardiovascular diseases, lower cancer incidence, and a decreased likelihood of neural tube defects.[42] Adequate folate levels particularly critical in pregnant women to prevent fetal neural tube defects. Folate deficiency is not uncommon among women of childbearing, especially during conception.[41,42] Vitamin B_{12} deficiency is likewise associated with a range of severe health issues, including anemia, neurological complications, and metabolic disorders.[43]

Several studies had linked folate and vitamin B12 deficiencies to the development of low incidence of macrocytic anemia in Saudi Arabia.[13,44] Macrocytic anemia was observed in 3.2% of adult patients in Abha city (involving 614 patients)[13] and in 2% of adult patients in Makkah city (involving 21,524 patients).[45] The current study did not find any association between low levels of folate and vitamin B_{12} with the occurrence of macrocytic anemia. Data regarding vitamin B_{12} and folic deficiency and the prevalence of macrocytic anemia among young adults in the Jazan region are scarce, with only a few studies conducted in Saudi Arabia.[13,33,45–49] Notably, no prior studies from the Jazan region have specifically investigated the prevalence of macrocytic anemia among young male and female students. As a result, the authors suggest a low occurrence of macrocytic in this demographic. This assertion is supported by two studies conducted in Jazan that focused on young male and female students (n = 134), as well as data from adults who underwent premarital screening (n = 1312), which did not identify any cases of macrocytic anemia.[10,11] Nationally, various studies have explored RBCs abnormalities and high MCV values in different regions of Saudi Arabia, including Taibah, Riyadh, Asser and Alghat.[33,47–49] The findings of the current study align with those reported in the Asser region, where no cases of anemia related to high MCV were documented.[48] Additionally, the prevalence of macrocytic anemia in Taibah and Riyadh was found to be low, at 0.4% and 0.7%, respectively.[33,47] However, it is worth noting that a recent study demonstrated a high prevalence of macrocytic anemia, which contradicts the findings of our study.[49] The observed discrepancy in the prevalence of folate and vitamin B_{12} deficiencies between studies could be attributed to variations in the populations studied and differences in sample sizes. Our research focused on healthy young adults, while the Alsagaby (2022) study included patients who had visited Prince Nasser bin Saad Al-Sudairy Hospital over a period of 12 months.[49] The presence of macrocytosis, as indicated by a higher MCV, was evident in elderly patients but not in younger individuals. Moreover, our study had a smaller sample size than those conducted in Abha and Makkah. It is worth mentioning that Saudi Arabia has implemented folate food fortification, which could contribute to the relatively low incidence of folate deficiency and its limited role in the development of macrocytic anemia.[39] This contrasts with the findings in different countries.[50,51]

In contrast to folate and vitamin B_{12} deficiencies, the study population exhibited low iron levels. It is important to note that the current study did not identify a significant coexistence of low levels of either folate or vitamin B_{12} with low levels of serum iron. Iron deficiency stands as one of the most prevalent common clinical ailments.[52] Factors such as increased iron demand and menstrual blood loss are the leading etiological contributors to ID.[52]

The present study, like many others, has certain limitations, including a relatively small sample size. Additionally, some participants were taken vitamin supplementation, which could potentially influence the results; however, this is consistent with practices in other studies. Moreover, this study focused on young adults, and other groups such as children, pregnant women, and the elderly were not assessed.

In summation, prevalence of low serum iron remains a subject of concern and interest, whereas macrocytic anemia, particularly attributed to deficiencies in folate and vitamin B_{12}, were not observed. While our study confirms the presence of these deficiencies, it does not reveal a direct association with macrocytic anemia, evoking intriguing avenues for future exploration. To advance our understanding, additional comprehensive studies encompassing diverse age groups, including children, pregnant women, and the elderly, are warranted. These inquiries should encompass a broad spectrum of sociodemographic variables and dietary habits and hold the key to addressing these challenges and enhancing the nutritional well-being of young adults in Saudi Arabia.

Ethical Approval

All procedures performed in the current studies involving human participants were approved by the Scientific Research Ethics Committee (REC-44/06/478), Jazan University and was conducted in accordance with the Declaration of Helsinki.

Informed Consent

Informed consent was obtained from all study participants.

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.

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Disclosure

The authors declare no conflict of interest.

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ORIGINAL RESEARCH

HIF2-α Expression in CML Patients Receiving Hydroxyurea Prior to Imatinib That Achieved Major Molecular Response (MMR) versus in Those Not Achieving MMR

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Introduction: Currently, Imatinib (IM) which is a Tyrosine Kinase Inhibitor (TKI), is the main treatment for patients with chronic myeloid leukemia (CML). Major molecular response (MMR) is used as therapeutic response. Resistance to IM may be caused by hypoxia which is regulated by hypoxia inducible factor (HIF) 2-α. The role of HIF2-α is currently not researched extensively. This study aimed to analyse the differences in HIF-2α expression between chronic phase CML patients that achieved MMR and those that did not achieve MMR.

Methods: This study used a cross-sectional method which analysed secondary data from whole blood samples in chronic phase CML patients aged 18–60 years that received hydroxyurea (HU) before IM, aged 18–60 years, received IM therapy for more than 12 months, and were willing to participate in the study. The exclusion criteria for this study were patients who were receiving IM at a dose of more than 400 mg/day. HIF-2α protein expression was examined using the enzyme-linked immunosorbent assay (ELISA) method. Differences between HIF-2α protein expression in groups that achieved MMR versus not achieving MMR was analysed using the Mann–Whitney test.

Results: A total of 79 subjects were obtained. The median HIF-2α was 90.56 pg/mg protein (3.01–4628.74). There was no statistically significant difference in expression of HIF-2α in the group that reached MMR and did not reach MMR, namely 123.45 pg/mg protein and 89.25 pg/mg protein respectively (p 0.718).

Conclusion: This study found no statistically significant difference between HIF-2α expression level and MMR achievement of chronic phase CML patients who received HU before IM therapy.

Keywords: CML, leukemia, imatinib, resistance, chronic myeloid leukemia, IM

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the excessive proliferation of myeloid cells at various stages of differentiation.[1] The disorder is characterized by a specific genetic alteration, a reciprocal translocation of chromosomes 9 and 22, which results in the formation of the Philadelphia chromosome.[1] This translocation leads to the fusion of the Breakpoint Cluster Region-Abelson murine Leukemia (BCR-ABL) gene, which plays a crucial role in the development and progression of CML.[1]

The effectiveness of CML therapy is evaluated by determining the level of therapeutic response, which is measured by major molecular response (MMR).[1,2] Other measurement of therapeutic response include complete hematologic response and

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complete cytogenetic response.[3] However, MMR is proven to be superior as outcome measurement, especially for predicting long-term relapse.[4]

Failure to achieve a therapeutic response can be caused by resistance to therapy.[2] The mechanisms of resistance can be either BCR-ABL dependent, caused by genetic mutations in the BCR-ABL gene itself, or BCR-ABL independent, which can be caused by various factors, including increased production of reactive oxygen species (ROS).[5–7] ROS play a significant role in maintaining genome integrity as they can damage DNA through direct or indirect processes. Research has also shown that ROS formation can occur as a result of hydroxyurea (HU) treatment before imatinib (IM) therapy.[6,7]

Hypoxia-inducible factor (HIF) plays a role in regulating oxygen homeostasis and is involved in the pathogenesis of various diseases, including leukemia. There are three isoforms of HIF protein: HIF-1α, HIF-2α, and HIF-3α.[8–10] HIF-1α is the most widely studied and is expressed in nearly all cell types. HIF-2α is expressed in a limited number of cell types, such as vascular endothelial cells, type 2 pneumocytes, renal interstitial cells, liver parenchyma, and myeloid cells. HIF-3α is currently less well-researched compared to the other isoforms.[8,11]

Increased expression of HIF has been reported in a majority of cases of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and CML.[12] In an in vitro study of HIF-2α expression in AML cases, it was found that there was an increase in HIF-2α protein expression which was associated with accelerated disease progression and poor response to therapy.[13] HIF-2α acts as a transcription factor and plays a role in cell proliferation. If the expression of HIF-2α increases, it is believed that the proliferation of CML cells will also increase.[14]

There are currently no studies that specifically examine the relationship between HIF-2α expression and treatment response in chronic phase CML. Therefore, this study aims to investigate the association between HIF-2α expression and response to therapy, as measured by an MMR in CML patients.

Method

This study was a cross-sectional study with a total sample of 118 blood samples (leukocyte protein isolates) saved from previous research subjects with the research title "Longer Hydroxyurea Administration Prior to Imatinib Mesylate is Risk Factor for Unsuccessful Major Molecular Response in Chronic-Phase Chronic Myeloid Leukemia: Possibility of P-Glycoprotein Role".[15]

This study recruited samples from chronic phase CML patients who received HU before IM at Hematology and Medical Oncology polyclinic at Cipto Mangunkusumo National General Hospital from January 2015 to June 2016. Procedures of BCR-ABL/ABL ratio is described and measured in previous studies.[14] Examination of HIF-2α expression was carried out by the enzyme-linked immunosorbent assay (ELISA) method.

In this study, MMR was measured after at least 12 months of IM therapy. Attainment of MMR was determined based on the most recent patient measurement, with a cutoff of BCR-ABL <0.1% defining MMR achievement.

Inclusion and Exclusion Criteria

The inclusion criteria for this study were chronic phase CML patients who received HU before IM aged 18–60 years, had received IM therapy for more than 12 months, and were willing to participate in the study. The exclusion criteria for this study were patients who were receiving IM at a dose of more than 400 mg/day.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., released 2019. IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.). Descriptive analysis of categorical data is presented as proportions (percentages). Continuous variables with a normal distribution are presented as means (average) with standard deviations (SD), while continuous variables with non-normal distribution are presented as medians and first and third quartile values except for HIF-2α and BCR-ABL ratio which use range. The Student's *t*-test was used to compare continuous variables with normal distribution, while the Mann–Whitney test was used for continuous variables with non-normal distribution. Correlation test used Spearman correlation analysis.

Ethics

This study was ethically approved by the ethics committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital with protocol number 2-0-8-0964. This study follows the guidelines from the Declaration of Helsinki. As this is a study using previously obtained blood samples from previous study, no informed consent was required based on evaluation by the ethics committee.

Result

From a total of 118 samples, 85 samples were found that met the inclusion criteria. Out of the 33 samples that were not eligible for inclusion, 2 were from patients in the accelerated phase, 10 were from patients over 60 years of age, 8 were from patients who had received IM therapy for less than 12 months, and 13 were from patients who had not been evaluated for an MMR. However, in total, only 79 samples were available for analysis, as the other 6 samples did not have sufficient blood volume for analysis.

The characteristics of the study subjects are shown in Table 1. The mean age of the CML patients in the study was 41.51 years. The median blast percentage of the patients in this study was 0% (0–1%), which is in accordance with definition of chronic phase CML from European Society of Medical Oncology.[2]

Of the total patients, 22 patients (27.8%) achieved an MMR. The median leukocyte count among our patients was 7000 mg/dL (5200–18,900). Median HIF-2α is 90.56 pg/mg protein (range: 3.01–4628.74) and the median BCR-ABL ratio was 4.77 (range: 0–94.35).

Table 1 Basic Characteristics of Research Subjects (N = 79)

Variables	Values
Age, years (SD)	41.51 ± 13.19
Male gender, (%)	43 (54.4%)
Hb, g/dL (SD)	12.20 ± 2.60
Leukocyte, /mL (Q1-Q3)	7000 (5200–18,900)
Trombocyte, 10^3/mL (Q1-Q3)	235 (160–331)
Basophil, % (Q1-Q3) Neutrophil, % (SD) Band neutrophil, % (Q1-Q3) Segmented neutrophil, % (SD) Lymphocytes, % (SD)	1 (0–3) 65.30 ± 15.03 0 (0–0) 65.23 ± 15.04 25.76 ± 13.05
Blast, % (Q1-Q3)	0 (0–1)
Hydroxyurea treatment duration, months (Q1-Q3)	3 (0–62)
≤ 6 months, (%)	60 (75.9%)
> 6 months, (%)	19 (24.1%)
IM treatment duration, months (Q1-Q3) 12–24 months, (%) 24–48 months, (%) 48–72 months, (%) MMR achievement Achieved MMR, (%) Did not achieve MMR, (%)	15 (12–72) 61 (77.2%) 13 (16.5%) 5 (6.3%) 22 (27.8%) 57 (72.2%)
HIF-2α, pg/mg protein (minimum-maximum)	90.56 (3.01–4628.74)
BCR-ABL ratio, international scale (minimum-maximum)	4.77 (0–94.35)

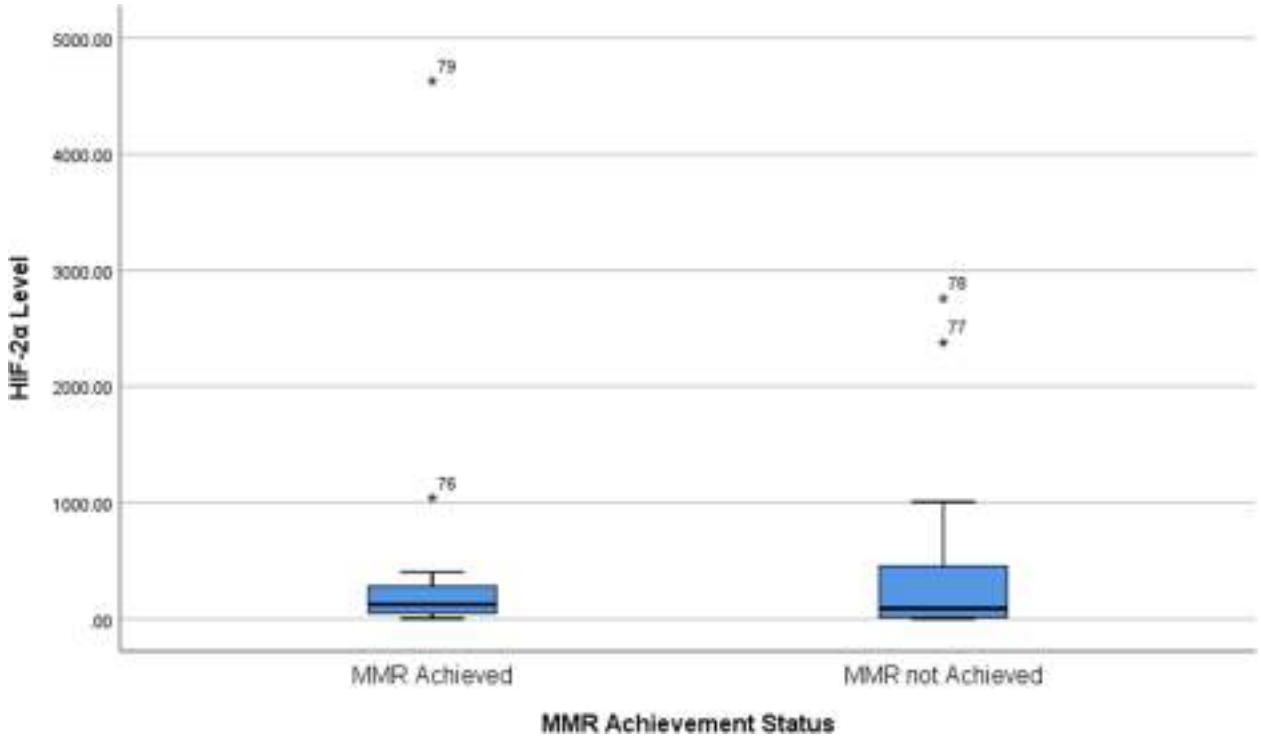

Figure 1 HIF-2α Level based on MMR status.

HIF-2 Alpha Expression and MMR Outcome

No statistically significant difference in median HIF-2α expression was observed based on the Mann–Whitney test between subjects who achieved MMR and those who did not (p-value: 0.718) (Figure 1). The median HIF-2α expression was 123.45 pg/mg protein (range: 3.01–4628.74 pg/mg protein) in subjects who achieved an MMR, while it was 89.25 pg/mg protein (range: 3.01–2755.7 pg/mg protein) in subjects who did not attain an MMR.

Further analysis is conducted that exclude patients receiving HU <2 months. A total of 44 patients receiving HU ≥2 months is available for analysis. Mann–Whitney test also showed no statistically significant difference in HIF-2α between the 2 groups (p: 0.235).

Correlation Between HIF-2α and BCR-ABL Ratio

Correlation analysis between HIF-2α and BCR-ABL ratio was conducted using Spearman correlation test. The correlation test showed weak negative correlation between the 2 variables but this is not statistically significant (correlation coefficient: −0.165; p value: 0.146).

Discussion

The mean age of the study population was 41.51 years, with the youngest participant being 19 years old and the oldest being 60 years old.[15,16] However, this mean age is lower when compared to the mean age of CML patients in Western countries, which tend to be higher. Research in the United States reported a median age of 66 years for CML patients. Studies in European countries such as France reported a median age of 56 years, while in Germany, it was reported as 57 years, in Sweden it was 60 years, and in the UK it was 59 years.[17–21] This difference in age may be attributed to racial and environmental factors.

In a review article that collected data from various registries in several countries in Asia such as China, India, the Philippines, Hong Kong, Singapore, Taiwan, South Korea and Thailand, the median age range was 36–55 years.[22] This was also in accordance with a study conducted by Mendizabal et al, who studied the relationship between geography and income and the age of patients when diagnosed with CML where the lowest median was in Asia and Africa, namely 47

years and the highest in the Oceania region, namely 72 years.[23] Thus, there may be an interaction between geographic factors and age. From this study, other hypotheses that need to be considered such as genetic mutations triggered by environmental exposures also emerged.

All subjects in this study had received IM mesylate therapy for at least 12 months. The median leukocyte level in this study was below 10,000/mL, namely 7000 (5200–18,900)/mL, with 42.4% (n: 31) subjects having levels leukocytes above 10,000/mL. Only 11 subjects still had blasts and the number of blasts above 5% was only observed in 1 subject. The median basophils in this study were 0.00 (0.0–3.0) % with a percentage of basophils above 20% observed only in 1 subject with a basophil level of 47%.

Several studies have shown that hypoxia inducible factors (HIFs) may play a significant role in CML progression by promoting stem cell survival, angiogenesis, and resistance to therapy.[24] A study by King Pan et al demonstrated that hypoxia-inducible factor 1 α (HIF1-α) is expressed in bone marrow of CML patients.[25] Interestingly, the Authors also demonstrated that HIF1-α is only partially suppressed by IM therapy. Thus, the Authors recommend anti-BCR-ABL1 and anti-HIF1- α to eliminate CML stem cells. Furthermore, a study by Hen Chen et al showed lack of colony formation in cells with HIF-1α knockdown.[14] In small cell lung cancer cells, IM may suppress angiogenesis.[26]

HIF-2α as a transcription factor increases the activity of the transcription factor c-Myc and the expression of cyclin D, both of which are required to maintain stem cells in an undifferentiated state.[27] HIF-2α is known to cause AML leukemia cells to survive the apoptotic process induced by endoplasmic reticulum stress.[28]

A study conducted by Forristal et al, showed that there was an increase in HIF-2α expression in AML cells compared to normal cells, and this increase in expression was associated with increased proliferation of leukemia cells, accelerated disease progression, and increased resistance to apoptosis.[13] Knockdown of HIF-2α in myeloid leukemia cell lines leads to decreased cell proliferation in vitro, slowing disease progression and prolonging survival.[13]

In this study, we did not find significant difference between HIF-2α expression level and MMR achievements in chronic phase CML patients. According to Deynoux et al there is conflicting data on the role of HIFs in leukemias.[12] Indeed, it is still not determined whether HIFs are oncogenes or tumor suppressors.

It is possible that HIF-2α may have no role in MMR at all in chronic phase CML but in blast phase CML instead. As proliferation of CML cells in chronic phase is relatively lower than in blast phase CML, which resembles AML, HIF-2α expression may not be different between groups that achieved and not achieved MMR. As described above, HIF-2α expression increase was associated with proliferation in AML.[13] It is also possible that IM treatment in both groups irrespective of MMR achievement may suppress HIF-2α expression and thus, causing no difference in HIF-2α levels. As a result, further studies are needed to elucidate the role of HIF2-α in CML and cancer in general. We also recommend further studies that assess HIF-2α expression in CML with blast phase and to compare HIF-2α expression between chronic phase and blast phase of CML. However, this may be difficult as patients with blast phase CML is very rare.

Study Limitations

Limitations of this study include variable months of measurement for MMR above 12 months and variable duration of HU treatment prior to IM.

Conclusion

This study found no statistically significant difference between HIF-2α expression and MMR in chronic phase CML patients who received HU before IM therapy. Further large-scale studies should be conducted to explore the role HIF-2 alpha in CML and leukemia.

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Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Establishment of Reference Intervals of Blood Parameters Among the Healthy Afghan Population

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Purpose: This study was designed and conducted to validate the reference values of hematological parameters for healthy adult male and female residents of Kabul city, Afghanistan.

Methodology: In this cross-sectional study, the samples were collected according to a non-random sampling method. Blood samples were collected from students and employees of Kabul University. The study included 166 males and 125 females, aged 18–45 years. The selection and exclusion of participants were carried out according to a questionnaire and the assessment of serum ferritin and vitamin B_{12} levels. Candidates with lower serum ferritin and vitamin B_{12}, a history of chronic disease, females with menstruation or pregnancy, and those with chronic abdominal pain were excluded.

Results: Reference ranges for all blood parameters were determined by a non-parametric method. The determined reference values were compared between males and females by the *Z*-test. Reference intervals for hemoglobin (4.5–6.3 g/dL for males and 3.66–5.67 g/dL for females) and hematocrit (36.23–55.93% for males and 30.20–53.86% for females) were significantly ($p<0.05$) higher in males. No significant ($p<0.05$) differences were observed between the reference intervals for the red blood cell count.

Conclusion: Therefore, we conclude that the commonly used reference intervals should be revised for the Afghan population, as our findings indicated higher reference values for the hemoglobin and hematocrit indices.

Keywords: reference range, blood parameters, Afghan population

Introduction

Successful therapy is the basic aim of a healthcare system. Scientists are working hard to invent new medicines and more effective treatment strategies. But all of these efforts can only be successful when there is an accurate diagnosis of the disease. For accurate diagnosis, along with the signs and symptoms of disease, medical laboratories play a pivotal role. Good laboratory practices ensure more accurate results. All of the strategies of good laboratory practices aim to establish the patient as a patient and the healthy individual as healthy. To achieve this aim, although the laboratory equipment, standards and qualified staff are important, the presence of correct reference ranges is equally important.[1]

The reference range or reference interval is the range of values of a specific analyte found in 95% of the healthy reference population. Historically, the term used was the "normal range", but according to ISO 15189, the use of the term normal range is now considered incorrect. This is because, first, 5% of healthy individuals do not meet the criteria or their results are outside the range, and second, "normal" is the term used for the normal distribution in statistics, but the distribution of findings in the reference interval is not always normally distributed.[2]

A number of factors can affect the results of any analyte while comparing it with the reference range. These include, age, sex, ethnic group, certain conditions such as pregnancy, athletic lifestyle, and environmental influences. Individual history and personal information are important to enable comparison of an individual's results with the right reference interval. Therefore, it is important that a distinct reference interval is used for each group of people.[2,3]

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An accurate reference interval is important not only for diagnostic purposes but also for screening purposes. In Afghanistan, anemia is a common problems in women of reproductive age and in children; therefore, screening tests are usually running continuously in different parts of the country to assess the need for new treatments and preventive strategies.[4] However, hematological parameters can be affected by different factors, such as age, body build, ethnic group, nutritional habits, and living environment, especially altitude.[5] It is therefore crucial to find out the reference ranges for the Afghan population.

In Afghanistan, medical laboratories use either the reference intervals from textbooks, usually originating in western countries, or the reference intervals provided by manufacturing companies, which are imported from different regions of the world. So far, there has been no validation of reference intervals for the Afghan population.[6] Thus, in this study, we investigated the ranges of hematological parameters among the Afghan population and compared them with the reference intervals.

Subjects and Method

The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. The methodology was reviewed and approved by the research committee of the Faculty of Pharmacy, Kabul University, and the academic council of the Faculty of Pharmacy, Kabul University. For validation purposes, we used the reference intervals of three well-known laboratories in Kabul city; each of these laboratories uses different reference intervals, although they cover the same population. Then, according to the CLSI guideline, we analyzed the blood samples of 20 reference individuals to analyze the validity of the existing reference intervals. More than 10% (more than two) were outside these reference intervals. We collected the blood samples of 20 more reference individuals, and the results of more than 10% fell outside the existing reference intervals. Thus, we decided to establish the reference intervals of hematological parameters for the Afghan population. This study is the first to be conducted in Afghanistan for the analysis of reference intervals of hematological parameters.

The study was designed as a cross-sectional study and conducted from October 2017 to March 2018. Sampling was performed using a non-random sampling method (convenience sampling), and subjects were selected from the male and female students and workers at Kabul University. Recruitment was carried out by placing written posters about the research in different parts of university. Informed written consent forms were signed by all volunteers, and they were interviewed using a written precoded questionnaire. Candidates with the following criteria were selected for sampling:

- Aged 18–45 years
- Do not have a chronic disease, eg, cardiovascular disease, hypertension, diabetes
- Have not had a blood transfusion in the past 3 months
- Have not donated blood in the past 3 months
- Do not have internal or external bleeding
- Females without pregnancy or menstruation
- Do not have a history of abdominal pain (a sign of intestinal parasites)
- Should not be a smoker or an addict.

Blood Sampling

Blood samples were collected using Vacutainers in two distinct tubes (EDTA and gel tubes, 3 mL in each) following standard phlebotomy techniques.

Determination of Blood Parameters

In blood samples obtained in EDTA tubes, the hemoglobin (Hb), hematocrit (HCT), red blood cell (RBC) count, and blood indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]) were determined using a Medonic hematology analyzer. The procedure was carried out three times for each test and the mean of all three results was selected. If a blood sample was damaged, the candidate was requested to give another blood sample.

Determination of Ferritin and Vitamin B_{12}

The blood samples obtained in gel tubes were centrifuged and then stored at −20°C until further analysis. On the day of analysis, the blood the serum was brought up to room temperature, and serum ferritin and vitamin B_{12} levels were determined by the ELISA method with an RT2100C microplate reader, following the manufacturer's guidelines.

Statistical Analysis

The data were arranged in Microsoft Excel and reference intervals were determined using MedCalc (MedCalc statistical software version 15.2). Reference intervals were determined by a non-parametric method; for data with a normal distribution, the reference interval was determined as the mean±2SD, and for data that were not normally distributed, the central 95% of numbers was selected as the reference interval.

Normality was assessed by the Shapiro–Wilk normality test, which is considered the best test of normality for the data frequency more than 50. For the determination of outliers, the method of Reed 1971 was used. Differences between males and females were analyzed by the *Z* test at a significance level of $p<0.05$.[7] The reference ranges are shown as box-and-whisker plots.

Results

In this study, 240 males and 198 females participated; 63 males and 54 females were excluded as a result of the questionnaire, and 11 males and 19 females were excluded because of low levels of ferritin. The level of vitamin B_{12} was normal in all participants. Therefore, the results of 166 males and 125 females were analyzed for determination of the reference ranges.

First, we determined the reference ranges for Hb, HCT, and RBC count for both the male and female population. We used the non-parametric method for the determination of the reference range; for normally distributed data, the reference range was selected as mean±2SD, and for data that were not normally distributed the reference range was determined as the middle 95% of data. We also compared the difference of reference ranges of each parameter for both groups of male and female to determine whether the difference was significant. According to the skewness, kurtosis, results of the Shapiro–Wilk test, and *p* values, HCT and RBC were normally distributed for the female population, and HCT was normally distributed for the male population, while the distribution of other parameters was not normal (Figure 1).

In the male population, the lowest recorded values were Hb 13.4 g/dL, RBC 4.3 million/µL, red blood cell distribution width (RDW) 9.6%, and HCT 34.1%, while the highest values were Hb 19 g/dL, RBC 7.2 million/µL, RDW 12.6%, and HCT 56.5%. According to the kurtosis, skewness, and Shapiro–Wilk test using MedCalc software, only the distribution of Hb was normal, and the reference ranges were determined by the non-parametric method. In female candidates, the lowest recorded values were Hb 9.3 g/dL, RBC 3.6 million/µL, RDW 7.9%, and HCT 26.5%, while the highest recorded values were Hb 18 g/dL, RBC 6.2 million/µL, RDW 14.5%, and HCT 56.3%. The results of these parameters are listed in Table 1.

According to the analyses of kurtosis, skewness, and the Shapiro–Wilk test, the data for all three parameters (Hb, RBC and HCT) in both male and female candidates, were not normally distributed (Table 1). The results for RBCs were normally distributed, while all other parameters rejected the normality test; hence, it was preferred to define the reference range according to the non-parametric method. Therefore, the reference intervals of Hb, RBC, and HCT were determined by MedCalc software as 90% reference intervals (Table 2).

Levels of blood indices were determined automatically using a Medonic hematology analyzer. For male candidates, the lowest values of blood indices were recorded as MCV 22.7 fL, MCH 22.7 pg, and MCHC 31.6 g/dL, and the highest recorded values were MCV 92 fL, MCH 33.8 pg, and MCHC 39.3 g/dL. For female candidates, the lowest recorded numbers were MCV 59.8 fL, MCH 19.9 pg, and MCHC 32.3g/dL, and the highest recorded numbers were MCV 97.4 fL, MCH 33.4 pg, and MCHC 32.3g/dL. The results are listed in Table 3. For the determination of reference ranges of blood indices, the same statistics were measured. All of the determined blood indices, MCV, MCHC, and MCH, rejected the Shapiro–Wilk test.

The reference ranges of the blood indices were determined by a non-parametric method using MedCalc software as 90% confidence intervals (Table 4).

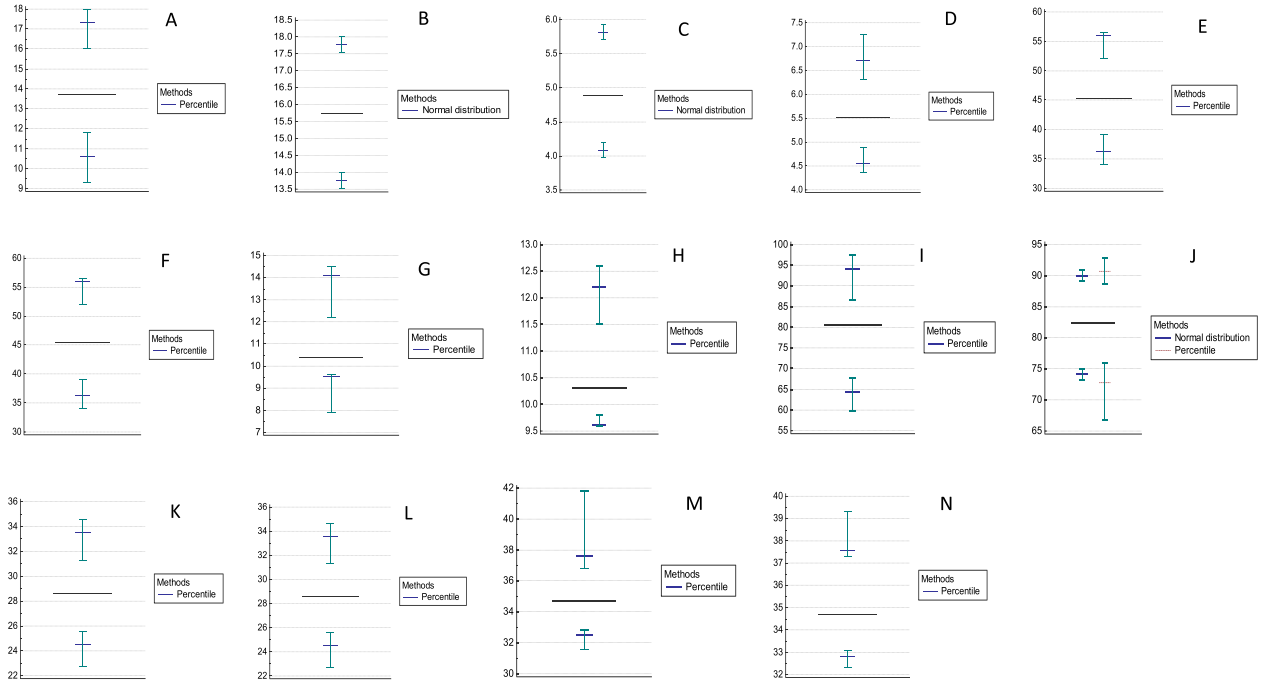

Figure 1 Box-and-whisker graphs of blood parameters obtained by MedCalc statistical software. (**A**) is belong to female hemoglobin, most of the data is between 11.9 to 16, (**B**) is belong to male Hb, the data is normally distributed therefore the length of whiskers are similar and from graph it is clearly can be observed that the reference interval is between 13.7-17.7, (**C**) is belong to the female RBCs the data is normally distributed and the reference range clearly observed from the middle point of whiskers. (**D**) is representing the RBCs of male candidates, the majority of data is situated between 4.9 to 6.5. (**E**) is belonging to the female HCT, most of the data is situated between 34 to 47 and the data is not distributed normally. (**F**) is representative of male HCT the data is not distributed normally and most of the data is situated between 38 to 52. (**G**) is belong to the female RDW, the data is not distributed normally, (**H**) is representative of male RDW, the data is not distributed normally. (**I**) represents female MCV, data is not distributed normally, (**J**) represents MCV for male population, (**K**) represents MCH for female population, (**L**) represents MCH for male population, (**M**) represents MCHC for female population, (**N**) represents MCHC for female population.

Discussion

The aim of reference values is to categorize an individual in a real-world health situation as being a patient or a healthy individual, or to show the real health situation according to the specific parameter; for example, if a person is suspected as having diabetes, the blood glucose level should really show the difference between the healthy individual and diabetic patients. According to the CLSI guideline (C28-A3), laboratories may adapt existing references that have been validated by donor laboratories or manufacturers. For this purpose, a laboratory should analyze a parameter of interest in a small

Table 1 Statistical Analysis of Blood Parameters for the Determination of Reference Intervals

	Hb		**RBC**		**HCT**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Sample size	125	166	124	166	125	166
Lowest value	26.5	34.1	3.66	4.37	9.3	13.4
Highest value	56.3	56.5	6.28	7.26	18	19
Arithmetic mean	39.7928	45.43	4.9535	5.54	13.8	15.7
Median	39.2	45.4	4.88	5.51	13.7	15.7
Standard deviation	4.489	3.89	0.4415	0.46	1.3	1.02
Shapiro–Wilk test for normal distribution	*W*=0.9317 (*p*<0.0001) Normality test rejected	*W*=0.9784 Normality test rejected (*p*=0.010)	*W*=0.9854 Normality test accepted (*p*=0.203)	*W*=0.9786 Normality test rejected (*p*=0.011)	*W*=0.96 Normality test accepted (*p*=0.00)	*W*=0.988 Normality test accepted (*p*=0.2)

Abbreviations: Hb, hemoglobin; RBC, red blood cell count; HCT, hematocrit.

Table 2 Lower Limit and Upper Limit of Reference Intervals of Blood Parameters, with 90% Confidence Intervals

	Hb		RBC		HCT	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Lower limit	13.7561	10.6000	4.5480	4.0838	36.2375	30.2000
90% CI	13.5290 to 13.9832	9.3000 to 11.8000	4.3700 to 4.8900	3.6600 to 4.3300	34.1000 to 39.1000	26.5000 to 34.4000
Upper limit	17.7740	17.3350	6.7082	6.0000	55.9300	53.8600
90% CI	17.5469 to 18.0012	16.0000 to 18.0000	6.3100 to 7.2600	5.6700 to 6.2800	52.1000 to 56.5000	47.0000 to 56.3000

Abbreviations: Hb, hemoglobin; RBC, red blood cell count; HCT, hematocrit.

Table 3 Statistical Analysis of Blood Indices for the Determination of Reference Intervals

	MCV		MCHC		MCH	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Sample size	125	166	124	166	125	166
Lowest value	59.8	66.7	32.3	31.6	19.9	22.7
Highest value	97.4	92.9	39.3	41.8	33.8	34.6
Arithmetic mean	79.8384	82.0849	34.9336	34.7313	27.912	28.5681
Median	80.5	82.4	34.7	34.7	28.1	28.6
Standard deviation	6.0887	4.0372	1.2449	1.314	2.4233	1.89
Shapiro–Wilk test	W=0.9260	W=0.9232	W=0.9697	W=0.9766	W=0.9260	W=0.9715
For normal distribution	Normality test rejected (p<0.0001)	Normality test rejected (p<0.0001)	Normality test rejected (p=0.0066)	Normality test rejected (p=0.0066)	Normality test rejected (p<0.0001)	Normality test rejected (p=0.0017)

Abbreviations: MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin concentration.

Table 4 Lower Limit and Upper Limit of Reference Intervals of Blood Indices, with 90% Confidence Intervals

	MCHC g/dL		MCH pg		MCVfl	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Lower limit	32.815	32.5	22.7	24.5225	64.4	72.745
90% CI	32.3000 to 33.1000	31.6000 to 32.8000	19.9000 to 23.3000	22.7000 to 25.6000	59.8000 to 67.9000	66.7000 to 76.0000
Upper limit	37.555	37.6	33.095	33.5375	94.2	90.7075
90% CI	37.3000 to 39.3000	36.8000 to 41.8000	31.0000 to 33.8000	31.3000 to 34.6000	86.6000 to 97.4000	88.6000 to 92.9000

Abbreviations: MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

sample size (n=20) of its covered population, and if no more than two (10%) fall outside the reference interval, the reference interval can be adopted; if more than two fall outside, then another 20 samples should be obtained, and then if no more than two fall outside the existing reference range it can be adopted; otherwise, the laboratory should establish a reference interval for its covered population.[8]

Several studies have been conducted and their results have shown that reference intervals are different for different populations. For example, the reference ranges for African-American, African and Afro-Caribbean populations are lower than for Caucasians.[5] Furthermore, climate and altitude are also known as factors having an effect on blood parameters.[9]

Kabul, the capital city of Afghanistan, is situated at an altitude of 1800 meters, and the population living in Afghanistanconsists of numerous ethnolinguistic groups: mainly the Pashtun, Tajik, Hazara, and Uzbek and several minorities.[10] Until now, no research has been conducted to verify whether the reference ranges which are used in laboratories in Kabul city are valid. Even the reference ranges used in laboratories in the same area were different from those of the manufacturers of their machines. This research is the first study undertaken in Afghanistan; however, similar research has been conducted in Iran, Pakistan, Iraq, Turkey, Africa, and Indonesia.

The reference range for RBC in our research was greater than those determined in Pakistan, Uganda, and South Africa, while it was similar to that in Turkey. Differences between the reference range of males and females were significant.[9,11,12]

The reference range of hemoglobin was significantly different ($p<0.05$) for males and females; therefore, we suggest different reference intervals for the two genders. The reference range of the hemoglobin level of the male Afghan population is higher than the results from populations in Pakistan and Uganda, while it was similar to the reference ranges of Turkey, Kenya, and South Africa. In the female population, the reference range was higher than in Pakistan and South Africa, but wider than in Turkey, Kenya, and Uganda, meaning that the lower limit is lower and the higher limit is higher than in these countries.[9,11]

The reference range for hematocrit was significantly different for the male and female populations, and it was higher than in Pakistan and Uganda and similar to those in Turkey, Kenya, and South Africa.[6,12,13] For RDW, the difference between the male and female populations was not significant; therefore, the same reference interval can be used for both sexes. RDW was measured only in the population from Turkey, and their reference range was higher than the Afghan population and also suggested the same reference interval for both sexes (Ozarda et al, 2017).[6] The reference range of MCV was not significantly different between the genders. In our study, the lower limit for the male population was higher than in Uganda and the higher limit was lower than in Uganda, and in the female population the reference range was lower than in Uganda. The reference ranges for East Kenya and South Africa were wider than our results.[6,1213] For MCH, the difference between the sexes was significant, and the reference range was lower than in Pakistan and was similar to that in Turkey. The difference in the reference range of MCHC was not significant between the sexes, and the results for Pakistan and Turkey were lower than our result.

Conclusion

The reference intervals for hematological parameters have not been validated in the Afghan population. Even well-known laboratories are using the reference ranges suggested by the manufacturers of machines and reagents. According to our results, these reference intervals are not accurate or valid for the Afghan population. We have established reference intervals for hematological parameters for the Afghan population, particularly those living in Kabul city. However, further studies are needed with a bigger sample size and random sampling method.

Disclosure

The authors report no conflicts of interest in this work.

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Survival and Treatment Outcomes of Childhood Acute Lymphoblastic Leukemia in a Low-Middle Income Country: A Single-Center Experience in West Java, Indonesia

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Survival and Treatment Outcomes of Childhood Acute Lymphoblastic Leukemia in a Low-Middle Income Country: A Single-Center Experience in West Java, Indonesia

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Purpose: This study aimed to determine the survival rates and treatment outcomes of patients with childhood Acute Lymphoblastic Leukemia (ALL) in a single-center study at Indonesia.

Patients and Methods: Factors contributing to the relapse and survival of ALL in Bandung, Indonesia, were evaluated. Data were collected from the medical record and the Indonesian Pediatric Cancer Registry (IPCAR). Subsequently, univariate and multivariate analyses were evaluated using Cox proportional hazard regression and Kaplan Meier was used for survival analysis. An analytic observational study was conducted on newly diagnosed children aged 1–18 with ALL from January 2019 to December 2022.

Results: A total of 137 children were included in the analysis, 30 (21,9%) were dropped out during treatment and 60.5% died during the study period. Most of the deaths occurred after relapse in 32 (38.5%) with a high early relapse (70.5%), occurring mainly during the maintenance phase (42.4%). At the one-year mark, the observed overall survival (OS) rate was at 36%, while event-free survival (EFS) was lower, at 19%. Univariate Cox regression analysis showed that the leucocyte counts at diagnosis (p=0.005) and response to induction phase ($p < 0.008$) was associated with the death of ALL. Furthermore, a response to induction phase was significant [hazard ratio 4.67 (CI 95%: 1.64–13.29); $p = 0.004$] in the multivariate analysis.

Conclusion: In conclusion, this study underscored the persistent challenges of high treatment discontinuation rates and the occurrence of very early relapses in low- to middle-income countries (LMICs), which significantly impacted the OS of children diagnosed with ALL.

Keywords: acute lymphoblastic leukemia, children, low-middle income country, relapse, survival

Introduction

Childhood cancer is a global concern that showcases varying survival rates depending on geographical location. In high-income countries, the survival of childhood cancer was more than 80%, while in low- and middle-income countries (LMIC) survival rate was only about 20%.[1] Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer, and its survival rate has significantly increased in the last two decades, with more than 90% in high-income countries. This has also improved in LMIC, although comprehensive data on global ALL survival in LMICs remain limited.[2] Several studies in Indonesia showed a survival rate of childhood ALL between 20% and 60%.[3–5]

The mortality of ALL primarily occurs during or even before the induction phase. Prognostic factors play a pivotal role in treatment risk stratification, predicting treatment outcomes, the possibility of failure, and the risk of relapse. The goal of treatment risk stratification is to enhance survival by increasing the intensity of therapy. Several known prognostic factors in ALL were age at diagnosis, gender, initial leucocyte count, mediastinal mass, central nervous system (CNS) involvement, and response to initial therapy or induction phase.[6–9] Minimal residual disease (MRD) using flow cytometry

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emerged as a strong predictor of outcome and relapse. However, MRD was not a routine examination due to limited facilities, particularly in Indonesia.[10,11]

Managing ALL in LMIC comes across challenges to improve outcomes and increase survival. To increase the survival of ALL, survival hinges on designed risk-based therapies rooted in prognostic factors that influence the outcome. Strategic selection of treatment intensity during the induction phase mitigates recurrence risks and minimizes treatment-related toxicity and death, while paying attention to compliance treatment to avoid treatment abandonment. This study aimed to determine the survival rates and treatment outcomes of children with ALL.

Materials and Methods

A retrospective cohort study was conducted from January 2019 to December 2022. This study involved newly diagnosed children ALL aged 1–18 years treated at the pediatric oncology division child health department, Hasan Sadikin Hospital, Bandung (provincial referral hospital in West Java, Indonesia). Patients falling under the categories of ALL L3, those above 18 years of age during the treatment period, or those who had passed away before commencing treatment were excluded. French-American-British classification was still used over the molecular/cytogenetic biomarkers of WHO classification due to unavailability of cytogenetic examination facility in our center.

The data collection process involved sourcing information from medical records and the Indonesian Pediatric Cancer Registry (IPCAR), including the demographic of the patients, nutritional status, presenting signs and symptoms, and date of diagnosis. The nutritional status was measured during the diagnosis phase and classified based on the WHO growth chart. Additionally, specific medical criteria were used for defining splenomegaly (enlarged spleen, Schuffner stage 1 or greater) and hepatomegaly (spleen positioned more than 2 cm below the arcus costae). Essential diagnostic factors such as the initial leucocyte count, chest X-ray, presence of leukemic blast in cerebrospinal fluid, and bone marrow morphology during diagnosis and evaluation after induction therapy were obtained. The evaluation of the induction phase was presented as remission if bone marrow morphology evaluation showed blast percentages of 5% or less. However, blast percentages exceeding this threshold were classified as induction failure. The patients' outcome was determined by relapse and remission during the evaluation after treatment. Specifically, relapse denoted the presence of leukemic blasts exceeding 20% in bone marrow morphology after patients had previously achieved complete remission. The timing of relapse was stratified into three categories: "very early relapse" if it occurred within 18 months of diagnosis, "early relapse" for relapses between 18 and 36 months, and "late relapse" for occurrences after 36 months post-diagnosis.

According to the Hematology Oncology Coordination Unit of the Indonesian Pediatric Society, the categorization of risk stratification was divided into groups, a standard risk (SR) and high risk (HR). High risk was characterized by an inadequate or poor response to prednisone defined by blast cell count ≥1000/μL found in peripheral blood at day eight after 7 days of prednisone and 1 dose of intrathecal methotrexate, absence of complete remission within six weeks with morphology examination, and meeting one or more of the subsequent criteria at the time of diagnosis: age <1 or >10 years old, leucocyte >$50x10^3$/μL, mediastinal mass more than 1/3 of the thoracic cavity, CNS involvement (presence of leukemic blast in cerebrospinal fluid), testicular involvement, mixed leukemia, T-cell as determined by immunophenotyping, while cases not meeting these conditions were classified as standard risk.

All patients were treated according to Indonesian Protocol ALL 2018 based on risk stratification (HR and SR). Patients monitoring occurred in the treatment course until the conclusion of the study period. Instances that were considered events for measuring event-free survival (EFS) encompassed relapse, treatment failure, or mortality. Patients who were selected to discontinue or withdraw from the study were accounted for until the last follow-up date. Statistical analysis for survival employed the Kaplan-Meier and was performed with SPSS IBM (Statistical Package for the Social Science Inc., Version 17). The factors affecting the survival of ALL were evaluated with univariate and multivariate analysis using Cox proportional hazard regression method with a confidence interval of 95% and $p<0.05$. This study was approved by the Ethics Committee of Hasan Sadikin General Hospital. All the data accessed has been authorized by Hasan Sadikin General Hospital for use in this study and kept anonymized using patient code.

Results

Study Population

This study identified 216 ALL children aged 1–18 years old with newly diagnosed ALL from January 2019 to December 2022. From all ALL patients identified in Hasan Sadikin Hospital, 31 had missing data. Only 185 patients were included, while 3 were diagnosed with bone marrow morphology of L3, 39 died before starting treatment, and 6 were transferred to another hospital. The data of the remaining 137 patients were subjected to analysis, as shown in Figure 1.

Patients Characteristics

The majority of subjects were male (52.2%), with a median age of 6 (1.0–17.7). Subsequently, nutritional status was normal (54.5%) in most subjects during diagnosis. A total of 83 (60.5%) children died until December 2022 and 32 (38.5%) of the cases occurred due to relapse. The majority of relapses transpired during the maintenance phase (42.4%), whereas occurrences during the induction and consolidation phases were observed in 4 (12.1%) and 5 (15.1%), respectively. The median time from the initial diagnosis to relapse was 15.38 months. Subsequently, most relapses occurred very early (<18 months) in 24 (70.5%) children, occurred early (18–36 months) in 7 (20.5%), and 1 (2.9%) child had late relapses. While 10 (7.3%) children completed therapy until the end of the maintenance phase and 2 children had a relapse after completing the treatment. The demographical and clinical characteristics of the subjects are shown in Table 1.

Survival Analysis

The cumulative overall survival (OS) and EFS rates at 1 year were 36% and 19%, respectively, as shown in Figure 2. In the survival curve, the inclusion of 30 children (21.9%) who dropped out during treatment was marked as censored data. The one-year OS rates for the standard risk (SR) and high-risk (HR) stratification groups were 54% and 29%, while the corresponding one-year EFS rates stood at 64% and 18% (Figure 3). Survival analysis with various demographic and clinical characteristics of subjects was carried out using the Cox regression method. Based on the results of univariate analysis, it was found that the initial leukocyte count ≥50x10^3/μL (p=0.005) and relapse during the induction-phase therapy evaluation (p<0.008) were associated with the death of ALL (Table 2). Moving to the multivariate analysis, the sole statistically significant variable was the absence of bone marrow remission post the induction phase, which carried a fourfold higher risk of death (Table 3).

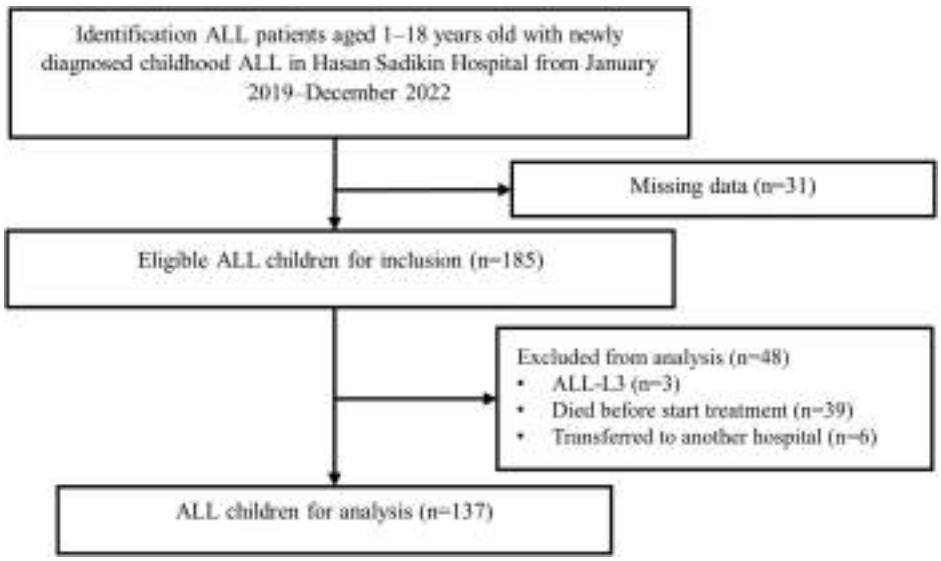

Figure 1 Flow chart of subject selection.
Abbreviations: ALL, acute lymphoblastic leukemia; ALL-L3, acute lymphoblastic leukemia with bone marrow morphology showed L3.

Table 1 Demographical and Clinical Characteristics of Subjects

Characteristic	N (%)
1. Gender	
Male	72 (52.2)
Female	66 (47.8)
2. Age at diagnosis (years)	
1–10	98 (71.0)
>10–<18	40 (29.0)
3. Nutritional status	
Normal	73 (54.5)
Mild malnutrition	9 (6.7)
Moderate malnutrition	44 (32.8)
Severe malnutrition	8 (6.0)
4. Leucocyte	
<50 x10^3/uL	100 (78.1)
≥50 x10^3/uL	28 (21.9)
5. Splenomegaly	
Yes	59 (58.4)
No	42 (41.6)
6. Hepatomegaly	
Yes	46 (44.7)
No	57 (55.3)
7. Mediastinal mass	
Yes	1 (1.0)
No	95 (99.0)
8. Risk Stratification	
HR	87 (64.0)
SR	49 (36.0)
9. Evaluation after the induction phase	
Failure to respond	7 (11.5)
Remission	54 (88.5)
10. CNS involvement	
Negative	85 (92.4)
Positive	7 (7.6)
11. Outcome	
Relapse	35 (55.5)
Remission	28 (44.5)

Abbreviations: HR, high risk; SR, standard risk; CNS, central nervous system.

Discussion

The result of this study showed 62 (59.7%) children with ALL died during and after chemotherapy, with a low cumulative OS rate. This is consistent with the previous studies conducted in several cities in Indonesia, which showed that the survival rate of ALL in children ranges from 20% to 60%.[12–14] The survival rate in low-middle-income countries based on CONCORD-2 data was 34.3–73.1%.[2,15] Neutropenia was the main cause of death-related treatment enhanced by infection, leading to life-threatening conditions requiring further treatment and may contribute to the delay of therapy during the induction phase.[16]

Most of the deaths occurred after children had relapsed (<18 months from diagnosis). This is consistent with previous studies in Indonesia and Latin America, showing that most deaths occur after relapse and most relapses occur very early.[17,18] Furthermore, a high rate of very early relapse indicated that the therapy given was inadequate to maintain remission and clearance of leukemic cells due to the contributions of several factors, such as delay of diagnosis and treatment, abandonment of therapy, and longer duration of the induction phase. Factors that contributed to the longer

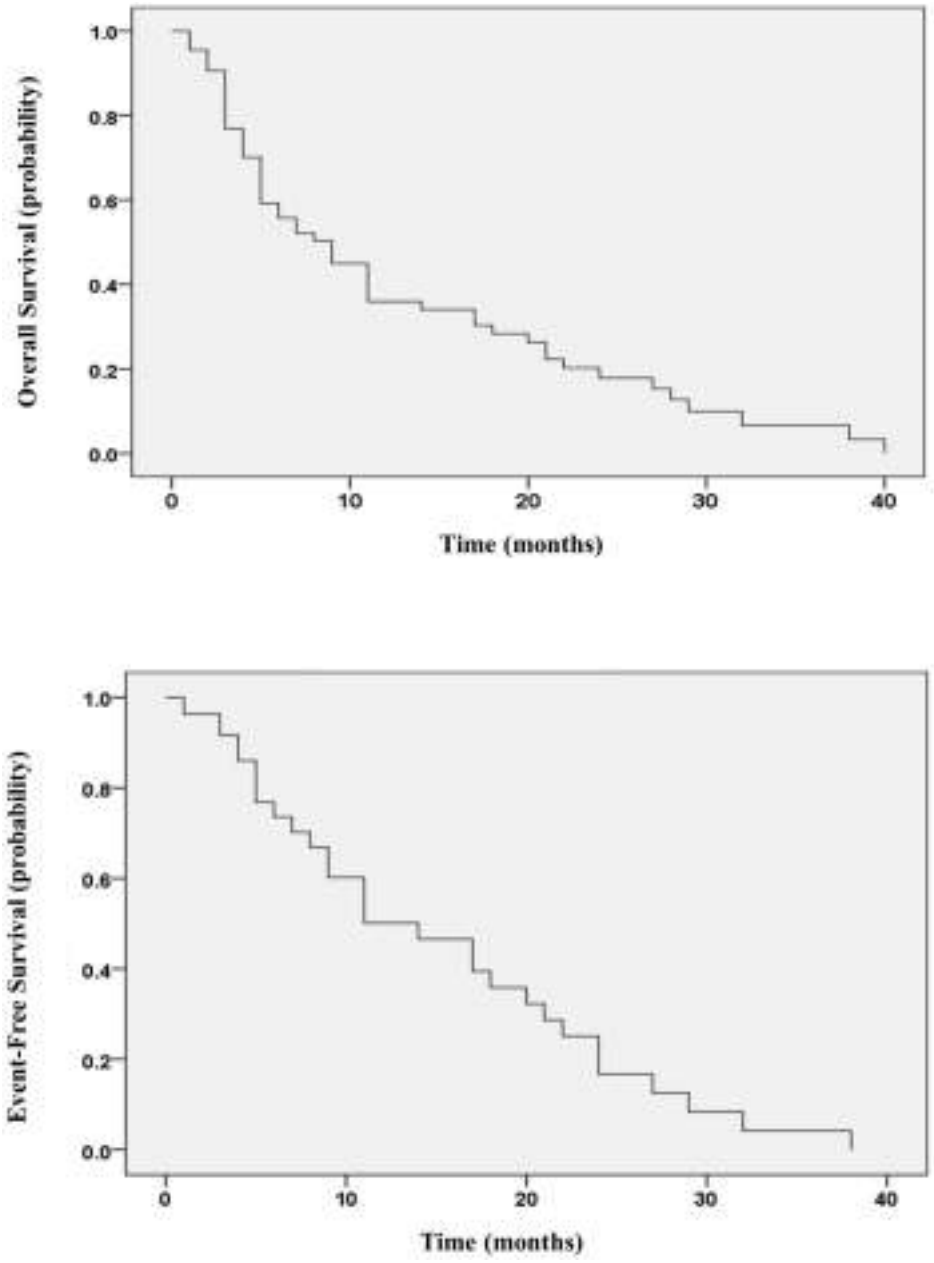

Figure 2 Kaplan–Meier curves of probable cumulative OS and EFS.

duration of the induction phase included infection during the induction phase, waiting for the chemotherapy room to be available, or side effects of previously administered chemotherapy. Inadequate clearance of leukemic cells during the induction phase might increase the risk of induction failure, as well as lead to a higher risk of relapse and severe complication.[19]

The result showed a high dropout rate of 21.9% due to treatment abandonment, which was still the main problem in developing countries, causing inadequate therapy and reducing the survival rate of ALL. Economic difficulties and lack of parental education might be barriers to therapy compliance despite the provision of national health insurance to cover all examination and treatment costs. Parents or guardians still needed to pay for daily living and the long duration of ALL treatment makes it difficult for parents to work and earn an income. Some were also hesitant to give chemotherapy to

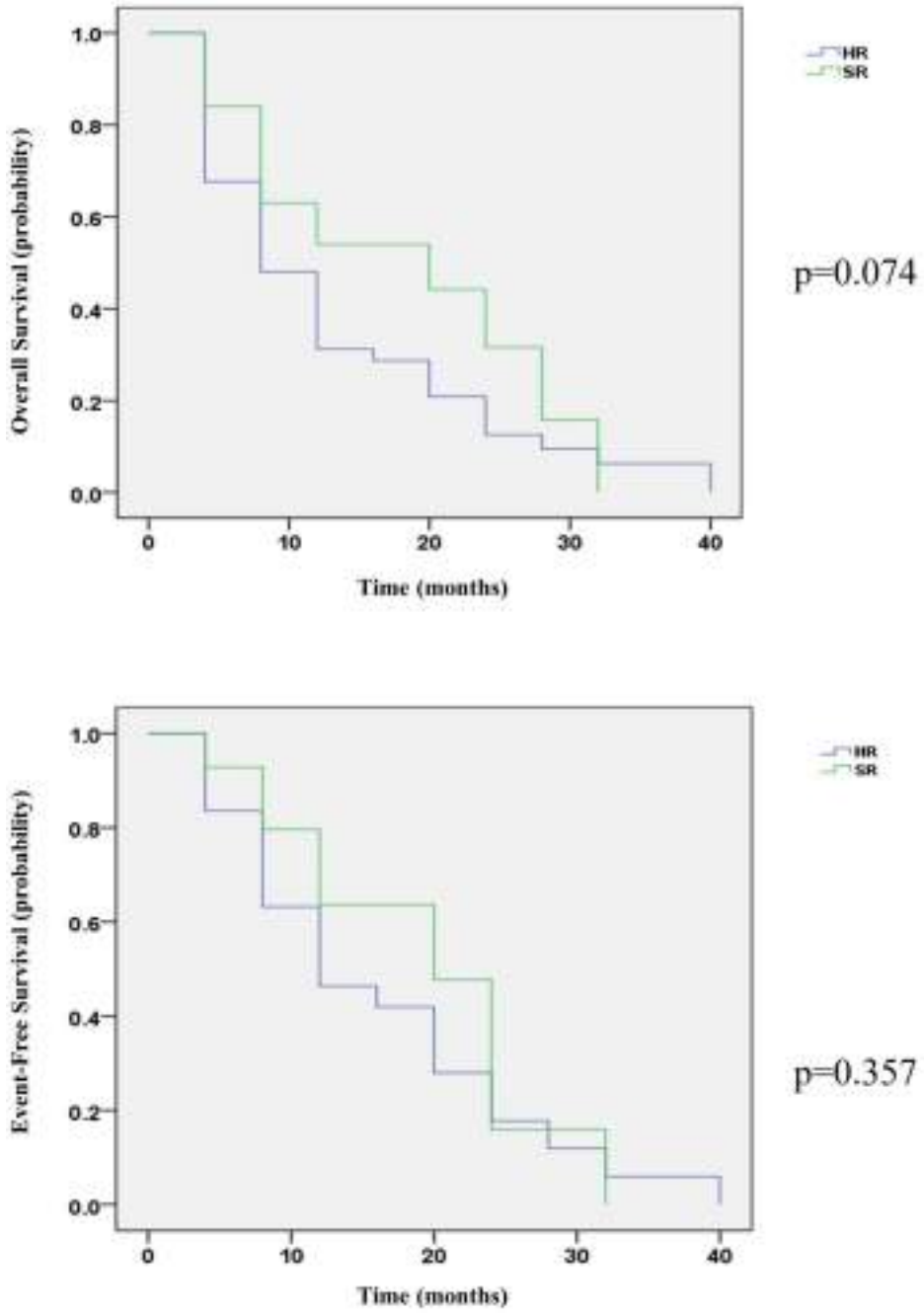

Figure 3 Kaplan–Meier curves of probable OS and EFS SR and HR risk stratification group.
Abbreviations: SR, standard risk; HR, high risk.

their children due to its severe side effects, long-term complications, and incurability beliefs.[6,7,17,20] Treatment abandonment should be fixed up in LMIC as one of the preventable factors of treatment failure in ALL.

Initial leucocyte count showed a significant result related to the outcome of ALL. The higher number of leucocyte counts at diagnosis showed the high proliferation of malignancy cells. Hyperleukocytosis leads to circulatory stasis due to the increased blood viscosity with blasts. This is related to the inflammatory process and the release of cytokines that cause ischemic tissue injury and damage by the entry of leukemic cells into the microcirculation.[21,22] A high initial leucocyte count was considered in the therapy risk stratification as one of the factors that worsened the prognosis. High-

Table 2 Association Between Demographical and Clinical Characteristics to the Outcome and Hazard Ratio to Overall Survival (OS)

Characteristics	Outcome		p-value*	Univariate Analysis	
	Survive	Death		Hazard Ratio for Death (CI 95%)	p-value
1. Gender			0.267		
Male	33 (53.2)	29 (46.8)		1.16 (0.69–1.94)	0.57
Female	25 (43.1)	33 (56.9)			
2. Age at diagnosis (years)			0.140		
1–10	47 (52.2)	43 (47.8)			
>10–<18	11 (36.7)	19 (63.3)		1.72 (0.99–1.94)	0.055
3. Nutritional status			0.825		
Normal	31 (50.8)	30 (49.2)			
Mild malnutrition	3 (50)	3 (50.0)		1.82 (0.54–6.04)	0.340
Moderate malnutrition	17 (41.5)	24 (58.5)			
Severe malnutrition	4 (50.0)	4 (50.0)			
4. Leucocyte			0.468		
<50 x10^3/uL	41 (48.2)	44 (51.8)			
≥50 x10^3/uL	10 (40.0)	15 (60.0)	0.641	2.44 (1.31–4.52)	0.005
5. Splenomegaly					
Yes	25 (49.0)	26 (51.0)		1.05 (0.56–1.95)	0.881
No	20 (54.1)	17 (45.9)	0.408		
6. Hepatomegaly					
Yes	19 (46.3)	22 (53.7)	1.00**	%1.%2 (0.56–1.85)	0.969
No	27 (55.1)	22 (44.9)			
7. Mediastinal mass					
Yes	0	1	0.004*	1.53 (0.21–11.33)	0.676
No	32 (41.6)	45 (58.4)			
8. Risk Stratification					
HR	28 (38.4)	45 (61.6)	0.045**	0.67 (0.43–1.04)	0.077
SR	30 (65.2)	16 (34.7)			
9. Evaluation after the induction phase					
Failure to respond	1 (14.3)	6 (85.7)	1.00*	4.00 (1.43–11.15)	<0.008
Remission	29 (58.0)	21 (42.0)			
10. CNS involvement					
Positive	3 (42.9)	4 (57.1)		0.54 (0.19–1.54)	<0.250
Negative	29 (39.7)	44 (60.3)			

Notes: *Chi-square test; **Exact fisher test.
Abbreviations: HR, high risk; SR, standard risk; CNS, central nervous system.

Table 3 Multivariate Cox Regression Analysis

Variable	β	SE (B)	p-value	Hazard Ratio Adjusted (CI 95%)
Initial model:				
Age at diagnosis (≥10–18 years)	−0.847	0.884	0.338	0.43 (0.08–2.42)
Initial leucocyte count (≥50x10^3/uL)	0.718	0.556	0.197	2.05 (0.69–6.10)
Risk stratification (HR)	0.275	0.382	0.471	1.32 (0.62–2.78)
CNS involvement (positive)	−1.543	1.265	0.222	
Evaluation after induction (failure)	1.806	0.676	0.008	6.09 (1.62–22.90)
End model:				
Evaluation after induction (failure)	1.541	0.534	0.004	4.67 (1.64–13.29)

Abbreviations: CNS, central nervous system; SE, standard error; HR, high risk; CI, confidence interval.

risk patients had lower OS and EFS as mentioned in many previous studies due to poorer prognosis and more intensive therapy.[18,23] Most of the patients were in the high-risk stratification group. In addition to the high rate of relapse, the delay in patients getting diagnosed and receiving treatment may lead to further progressivity of leukemic cells requiring the administration of more intensive therapy.

Early response to induction-phase therapy was a significant predictive factor in the outcome of children with ALL. Patients who have induction failure often experience disease progression to become more severe and even cause death. Steroid response, morphological assessment, and MRD evaluation strongly predict therapy outcomes guide the adjustment of treatment intensity and estimate the likelihood of relapse. Response to steroids, morphological assessment, and MRD assessment are strong predictors for predicting therapy outcomes, adjusting the intensity of therapy given, and estimating the likelihood of relapse. In several developing countries with limited MRD facilities, morphological examination at the end of the induction phase still plays an important role in the assessment of ALL therapy.[24,25] The longer duration of the induction phase could worsen the prognosis and the outcome of therapy, as well as increase the risk of failure. Induction phase therapy was the most important because it eliminated all leukemic cells, while other phases prevented the risk of relapse and maintained EFS.[8]

The limitations of this study were in its retrospective design and conducted at a single center, potentially resulting in low external validity. In addition, the high dropout rate means that children cannot be followed until the end of therapy or until relapse occurs. Early death before starting treatment was caused by late of diagnosis and some had refused to referred to tertiary hospital. Many variables showed insignificant results in terms of ALL therapy in this study, as it might be influenced by the small number of subjects investigated. Future studies are expected to assess characteristics that may influence the survival of ALL in LMIC, such as socio-economic factors, parental education, family perception of the disease, and distance between home and health facilities. This assessment should be incorporated into the prospective cohort design of the study.

In conclusion, the evaluation conducted after the induction phase by bone marrow morphology remained a remarkable predictor for the outcome of ALL therapy, specifically in limited resources settings. Treatment delay and abandonment also played an important role in the outcome of ALL in LMIC. Furthermore, improving the survival of ALL remained a challenge in LMIC to determine optimal treatment intensity with less treatment-related toxicity and maintain compliance, as well as reduce delay of diagnosis and treatment.

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Disclosure

The authors declare no conflicts of interest in this work.

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Magnitude, Associated Factors and Morphological Types of Anemia Among Hospitalized 6–59 Months Age Children at Jimma Medical Center, Southwest Ethiopia – A Hospital-Based Cross-Sectional Study

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ORIGINAL RESEARCH

Magnitude, Associated Factors and Morphological Types of Anemia Among Hospitalized 6–59 Months Age Children at Jimma Medical Center, Southwest Ethiopia – A Hospital-Based Cross-Sectional Study

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Background: Anemia is among the major public health problems that cause significant morbidity and mortality among children around the world. Anemia in children of age 6 months to 5 years is a major health problem in most developing world countries with estimated prevalence of about 43%.

Objective: To determine the magnitude, associated factors and morphological types of anemia among hospitalized 6–59 months age children from June 15 to October 15, 2022 at Jimma Medical Center, southwest Ethiopia.

Methodology: Hospital-based cross- sectional study design was conducted from June 15 to October 15, 2022 at Jimma Medical Center, involving 383 hospitalized children aged 6–59 months by employing convenient sampling technique. Data of sociodemographic characteristics and other associated factors of the study individuals waere collected using a pre-structured questionnaire. Clinical data were collected by physical examination and from history of client by medical interns and nurses. Then 3 mL venous blood was collected and analyzed for complete blood count. Data were coded, cleared and entered into EpiData version 4.6 and exported to SPSS version 25 for analysis. Bivariable and multivariable binary logistic regression was used to identify associated factors.

Results: The overall prevalence of anemia among hospitalized 6–59 months age children was 57.2%; out of them 30.82% were moderate. In the present study children with malaria infection, AOR = 1.15 (95% CI: 0.017, 0.781), Cchildren with severe malnutrition, AOR = 2.046 (95% CI: 0.306, 1.366), and children with low family income, AOR = 2.6 (95% CI 0.475, 0.894) were independent variables associated with anemia.

Conclusion and Recommendation: Anemia among study participants is found to be a severe public health problem. Based on this finding, more intervention is needed with health education on nutrition and child feeding.

Keywords: anemia, magnitude, 6–59 months, morphological types, southwest Ethiopia

Background

Anemia is defined as a decrease below the reference range for healthy people of roughly same age, sex, and race, under the same environmental conditions, in hemoglobin (Hgb) concentration, hematocrit, or the number of red blood cells per liter.[1]

Anemia is most prevalent micronutrient deficient disarray, which can affect a person at all stages of life at any time, particularly children of 6–59 months and pregnant women due to their increased need for iron, a micronutrient.[2] The World Health Organization (WHO) defines anemia depending on age and sex based on the typical amounts of hemoglobin: children 6 month to 5 years old = 11g/dl, children aged 6–14 years = 12g/dl, adult men >15 years = 13g/dl, expectant mothers = 11g/dl and women who are not pregnant = 12g/dl.[3]

The size of red blood cells (RBCs), as determined by the mean corpuscular volume (MCV), is typically used to categorize anemia or classified morphologically through peripheral blood smear examination based on hemoglobinization of red blood

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cells (RBC) as normochromic, characterizing red blood cell coloring normally, and as observed when 2/3rds of a red cell is hemoglobin, or hypochromic, characterized by light red cell coloring and an increase in the central pale region.[4,5]

The etiology of anemia is complex and multifactorial; it is correlated with biological, socioeconomic, and environmental factors such as intestinal parasite infestations, malaria, HIV infection, wasting, low dietary diversity, food scarcity, timely initiation of supplemental feeding, hematological malignancies, persistent diseases such as sickle cell disease (SCD), maternal weight, and prenatal care visits.[6–8]

One of the main public health issues affecting children worldwide is anemia, which significantly increases morbidity and mortality rates.[9] One of the biggest global public health concerns, it has a significant impact on social and economic growth as well as human health in both developed and developing nations.[9,10] Although its prevalence has decreased, it is remains a major global public health concern.[11] According to new demographic and health survey (DHS) report cycles between 2005 and 2018, the prevalence of anemia among children aged 6 to 59 months was more than 40% in the majority of developing nations with low and intermediate incomes, and it is categorized as a serious public health issue.[12] Sub-Saharan African nations such as Kenya 48.9%,[13] Mali 55.8%,[14] Tanzania 79.6%,[15] and Ethiopia 57%[16] have terrible problems.

Methods

Study Area

The study was conducted at JMC which is found in Jimma town. The town is located in the southwestern part of Ethiopia, 345 km from Addis Ababa, the capital city of Ethiopia. The geographic coordinates of the area are approximately 7° 40'N latitude and 36° 50'E longitude with an altitude of 1,780 meters above sea level. Based on the 2007 census conducted by CSA, the total population of the zone is 2,486,155 of whom 1,250,527 are men and 1,235,628 are women. Jimma Medical Center (JMC) is one of the oldest hospitals in Ethiopia and it is the only teaching and referral hospital in southwest Ethiopia, with 800 bed capacity and a catchment population of over 15 million people.

Jimma Medical Center laboratory provides a laboratory service for both inpatients and outpatients. The laboratory is now departmentalized into seven major sections known as Central Processing Unit, Parasitology, Hematology and Parasitology, Microbiology, Clinical Chemistry and Urine Analysis, Serology (Immunology) units, general bacteriology and molecular biology. Moreover it has two site labs namely, CD4 testing site lab and emergency test site lab. Jimma Medical Center provides a service for around 2,544 children annually.

Study Design and Period

A hospital-based cross-sectional study was conducted to determine the magnitude and associated factors of anemia among hospitalized children aged 6–59 months at JMC, southwest Ethiopia from June 15 to October 15, 2022.

Population

Source population

All 6–59 months old hospitalized children at Jimma Medical Centre.

Study population

All 6–59 months old hospitalized children whose parents or guardians consented to participate in the study at JMC during the study period and who fulfilled the selection criteria were the study population.

Inclusion and Exclusion Criteria

Inclusion Criteria

All children whose age was between 6–59 months and whose parent or guardian was willing to give information during the study period was included into the study.

Sample Size

The minimum sample size required for analysis was determined by using single population proportion formula,

$$n = (Z\alpha/2)^2 \frac{p(1-p)}{d^2}$$

Where n= sample size, z = statistic for a level of confidence (z =1.96 at 95% CI), p = expected prevalence or proportion by taking 48.9%,[17] d = margin of error (if 5%, d= 0.05).

$$n = \frac{(1.96)^2 * (0.489) * (1-0.489)}{(0.05)^2}$$

n = 383

The final minimum sample size was 383.

Sampling Technique

The convenient sampling technique was used until the required sample size was achieved.

Variables

Dependent variables

- Anemia

Independent variables

- Sex and age of child (months)
- Residence of family
- Annual family income
- Mean Upper Arm Circumference (MUAC)
- Diagnosis at admission
- Intestinal parasites infection
- Household food insecurity
- Malaria infection
- Children <5 years in the house
- History of chronic disease
- Habit of tea drinking

Materials Required

- 70% alcohol
- Cotton
- Gauze
- Glove
- Gown
- Marker
- Microscope
- Oil immersion
- Slide
- Syringe
- Test tube
- Wright stain

Data Collection Techniques and Instrument

Based on a signed consent form by guardians, the children who met the requirements for inclusion were added to the study. Sociodemographic details include the children's age, sex, place of residence, and history of chronic disease. Additional information was asked on family monthly income, and clinical conditions and additional study-related factors. Data were gathered by six nurses and medical interns working in the children's department using a pre-structured questionnaire, direct interviews with the guardian, and information from their medical history. Nine questions were used to gather information on household food insecurity, and based on the answers, the situation was classified as either light, moderate, or severe (Annex-1 section-5).

MUAC of children was measured by measuring the circumference of the left upper arm at the mid-point between the tip of the shoulder and the tip of the elbow, using a measuring or MUAC tape having spring tension attachment at the mid-upper arm in millimeters (mm) or in cm. The cut-off points for classification of nutritional status was according to Nutrition Assessment, Counseling, and Support (NACS).[18]

Sample Collection

Three milliliters of venous blood was collected aseptically by the nurses and it was then transported to hematology unit for hemoglobin, other red blood indices determination (Annex-2), thick and thin blood film was made for hemoparasite (malaria) investigation and species identification, respectively, and for anemic clients thin smears were made for morphological study. Stool sample was collected by giving collection cup and direction to guardians than transported to parasitology unit for parasite examination.

Laboratory Analysis

Hemoglobin and other red blood cell indices was measured by 800 DHX Beckman Coulter (Danaher Corporation, United States) machine by photometric and electrical impedance method,[19] (Annex-2) then the child's hemoglobin level was classified as serious anemic if it was less than 7 g/dl and as anemic if it was less than 11 g/dl. The morphological types of anemia were done by making a thin blood smear then staining the smear by Wright staining and thick and thin blood film was made and stained with Giemsa for malaria investigation and identification of malaria, respectively (Annex-3 and 4). Parasite examination was done by laboratory technology working at JMC parasitology unit by making stool smear by wet mount method and identification of parasite was made by using 10x or 40x objective (Annex-4).

Data Quality Assurance and Control

Before data collection and the actual study was conducted, colleagues reviewed the questionnaire to ensure the quality of the data. They determined whether or not the questionnaire was acceptable and whether or not it had the essential information. If not, they made any necessary modifications. Throughout the data collection period, data collectors received training and regular monitoring.

Standard operating protocols were followed throughout specimen collection and CBC analysis to ensure the quality of the laboratory results (Annex-2). Therefore, blood was distributed to the test tube wall and well mixed by gently inverting the tube 8–10 times in order to prevent hemolysis following collection. Samples were examined to make sure they meet the requirements, which include sufficient volume, proper clotting, hemolysis, and collecting time. Labeling was done on the sample and the request paper with the same identifying number to prevent confusion following collection. The quality of blood film made was checked to whether it met acceptable criteria i.e. not too thick, not too long, free from lines and holes, has a smooth tail, whether the slide is labelled with code and well stained. The quality of stool smear was checked for acceptable criteria.

To reduce background error, a daily background run was conducted. Prior to analyzing the patient's sample, the reagent's expiration date was verified, and the necessary internal quality controls were performed before sample assaying. Daily cross-checking of gathered data with records and on-site supervision of the data collector throughout the data collection period served to preserve the quality of the sociodemographic and clinical data. The test findings were not shared with anyone. Every lab test outcome was documented, reported, and specimens were handled carefully.

Data Analysis

After being cleaned, updated, and carefully verified for completeness, the data from the laboratory and the questioner was put into EpiData 4.6. After that, it was moved into a version 25 statistical program for social sciences so that it could be examined.

Prior to any analysis, the Shapiro–Wilk and Kolmogorov–Smirnov tests were used to confirm that the data were normally distributed. Standard deviation, mean, frequency, and percentage were used to characterize a descriptive result. Tables and charts were also used to present the results. A bivariate and multivariate logistic regression analysis was conducted to evaluate the contributing components. When the P-value in the bivariable binary logistic regression was less than or equal to 0.25, the multivariate logistic regression was examined using the backward stepwise likelihood ratio. Through the computation of the odds ratio with a 95% confidence interval, the relationship between the independent variable and the categorical outcome variable was assessed. The Hosmer and Lemeshow test was used to assess the model fitness of the final logistic regression model at a p-value of more than 0.05. Ultimately, a P-value of less than 0.05 was deemed statistically significant.

Results

Sociodemographic Characteristics of Study Participants

Altogether, 383 participant data were included in the study. From this 55.4% (n = 212) of studied children were males. The mean age was 28.44 ±1.48 months. Children below one year old constituted 98 (25.6%) of the study population. About 57.7% (n = 221) were from urban area in residence. About 46.4% (n = 178) of study participant families were low income level (less than 3000 ETB) (Table 1).

Table 1 Socio-Demographic Characteristics of Hospitalized Children of 6–59 Months Age and Their Parents at Jimma Medical Center, Southwest Ethiopia from June 15 to October 15, 2022

Characteristics of Participant	Categories	Frequency	Percent
Sex of child	Male	212	55.4%
	Female	171	44.6%
Age of child in months	6–11 months	98	25.6%
	12–23 months	91	23.8%
	24–35 months	44	11.5%
	36–47 months	73	19.1%
	48–59 months	77	20.1%
Residence	Urban	221	57.7%
	Rural	162	42.3%
Children <5 year in house	1	91	23.8%
	2	173	45.2%
	3	103	26.9%
	4	16	4.2%
Family income in ETB (Ethiopia birr)	High	100	26.1%
	Middle	105	27.41%
	Low	178	46.4%

Notes: High= >7500 ETB, Middle =3000–7500 ETB, Low= <3000 ETB. Currency conversion rate −1USD = 51.9 ETB.

Health Status and Anthropometry Measurement of Child

Regarding the primary reason for admission, about 30.3% (n = 116) and 29.5% (n = 113) participants were admitted to hospital because of SAM and pneumonia, respectively. From the total, only 9.66% (n = 37) participants were positive for malaria and among the infected participants 75.67% (n = 28) were due to *Plasmodium vivax*. Additionally only 9.9% of study participants were positive for intestinal parasites. Near to half of the study participants were in normal range for MUAC measurement which were 44.5% (n = 172) (Table 2).

Knowledge Towards Child Feeding and Household Food Insecurity Access Scale Data

The assessment of knowledge towards child feeding showed that about 71.3% (n = 273) study participant had habit of tea drinking, and out of them, 89.4% (n = 244) were drinking tea along with their meal. A little over 36.3% (n = 139) of children were born and raised in food-secure homes, while 35.5% (n = 136) were born and raised in food-insecure households, according to the assessment of household food insecurity access scale (Table- 3).

Table 2 Health Status and Anthropometric Measurement of Hospitalized Children of 6–59 Months Age at Jimma Medical Center, Southwest Ethiopia from June 15 to October 15, 2022

Health Status of Child	Categories		Frequency	Percent (%)
Diagnosis at admission	Pneumonia		113	29.5
	SAM (severe acute malnutrition)		116	30.3
	Meningitis		30	7.8
	Septicemia		40	10.44
	Gastroenteritis		32	8.35
	Complicated pertussis		30	7.8
	UTI (urinary tract infection)		10	2.6
	Other		12	3.13
History of chronic disease	YES		47	12.3
	NO		336	87.7
Malaria infection	NO		346	90.34
	YES		37	9.66
		P. vivax	28	75.67
		P. falciparum	9	24.33
Parasite infection	NO		345	90.9
	YES		38	9.9
		Giardia	15	39.47%
		S. mansoni	9	23.68%
		Ascaris	9	23.68%
		Hookworm	5	13.17%
MUAC (mid-upper arm circumference)	Normal		172	44.5
	Moderate		72	18.79
	Severe		139	36.29

Table 3 Knowledge Towards Child Feeding and House Food Insecurity Access Scale Data of Hospitalized Children of 6–59 Months Age and Their Parents at Jimma Medical Center, Southwest Ethiopia from June 15 to October 15, 2022

Data		Frequency	Percent (%)
Habit of tea drink	No	215	56.13
	Yes	168	43.86
	Before meal	2	1.5
	Along meal	150	89.4
	After meal	15	9.1
Household food insecurity access scale	Food secure	139	36.3
	Mild food insecure	56	14.6
	Moderate food insecure	52	13.6
	Severe food insecure	136	35.5

Prevalence and Severity Pattern of Anemia Among Study Participants

Male and female prevalence of anemia varied, with the total prevalence of anemia among children aged 6–59 months being 57.2% (219/383) (Table- 4).

Among the anemic study participants 18.53% (n = 71), 31.1% (n = 119) and 7.57% (n = 29) had mild, moderate and severe anemia, respectively (Figure 1).

Table 4 Bivariate and Multivariate Logistic Regression for Magnitude of Anemia and Associated Factor Among Hospitalized Children of 6–59 Months at Jimma Medical Center

Variable	Categories	Anemia		COR 95% CI	AOR 95% CI	p-value
		Yes (%)	No (%)			
Age of child	6–11	60(15.6%)	38(9.92)	1		
	12–23	57(14.89)	34(8.87)	0.88(0.185,2.237)		
	24–35	24(6.26)	20(5.2)	1.29(0.161,2.031)		
	36–47	39(10.18)	34(8.87)	1.38(0.185,3.75)		
	48–59	39(10.18)	38(9.92)	1.55(0.236,3.351)		
Residence	Urban	115(30.03)	106(27.66)	0.605(0.706,3.872)	0.94(0.243, 3.62)	0.98
	Rural	104(27.17)	58(15.14)	1	1	
No of <5 year children	1	42(10.97)	49(12.79)	1.2(0.403,3.318)		
	2	92(24.03)	81(21.14)	0.88(0.316,2.45)		
	3	77(20.12)	26(6.78)	0.33(0.115,0.99)		
	4	8(2.09)	8(2.09)	1		
Sex	Male	128(33.43)	84(21.9)	0.713(0.309,1.647)		
	Female	91(23.77)	80(20.6)	1		

(*Continued*)

Table 4 (Continued).

Variable	Categories	Anemia		COR 95% CI	AOR 95% CI	p-value
		Yes (%)	No (%)			
Diagnosis at admission	Pneumonia	67(17.58)	46(12.09)	0.55(0.059,9.04)		
	UTI	8(2.1)	2(0.52)	0.4(0.113,4.685)		
	Septicemia	17(4.39)	8(2.19)	0.4(0.082,25.812)		
	Gastroenteritis	16(4.17)	16(4.17)	0.8(0.228,2.078)		
	SAM	80(20.87)	36(9.39)	0.38(0.595,22.23)		
	Pertussis	8(2.19)	21(5.49)	2(0.545,6.671)		
	Other	8(2.1)	4(1.04)	1		
Malaria result	Yes	22(5.7)	15(3.91)	0.27(0.037,1.06)	1.15(0.017,0.781)	0.001*
	No	197(43.96)	149(38.90)	1	1	
Intestinal parasite	Yes	21(5.49)	17(4.39)	0.9(0.233,3.721)		
	No	197(51.64)	147(38.46)	1		
MUAC	Normal	84(21.97)	88(23.08)	1	1	
	Moderate	38(9.89)	34(8.79)	0.84(0.273,2.62)	2.12(0.525,8.618)	0.045*
	Severe	97(25.27)	42(10.98)	0.41(0.158,1.084)	2.046(0.306,1.366)	
Tea drink habit	Yes	139(36.26)	29(7.69)	2.6(0.974,7.11)		
	No	80(20.88)	134(35.1)	1		
Family income	High	50(13.18)	50(13.18)	1	1	
	Middle	46(12.09)	59(15.38)	1.2(0.414,3.917)	1.64(0.301,9.01)	0.043
	Low	123(32)	55(14.28)	0.44(0.159,1.26)	2.6(0.475,0.894)	
HFIAS	Food secure	72(18.7)	67(17.58)	1		
	Mild insecure	25(6.59)	29(7.69)	1.2(0.342,4.48)		
	Moderate insecure	38(9.89)	18(4.69)	0.47(0.121,1.84)		
	Severe insecure	84(21.98)	50(13.18)	0.63(0.237,1.71)		

Abbreviations: HFIAS*, Household food insecurity access scale; UTI, urinary tract infection; MUAC, mid-upper arm circumstance; SAM, severe acute malnutrition; COR, crude odds ratio; AOR, adjusted odds ratio, CI-confidence interval.

Morphological Classification of Anemia and Severity Pattern of Anemia Among Study Participants

Based on blood film and MCV results, the majority of our participants with anemia had normocytic normochromic blood picture 37.1% (n = 142), microcytic hypochromic were 15.7% (n = 60), and macrocytic normochromic blood picture 4.4% (n = 17) (Figure 2).

Factors Associated with Anemia Among Study Participants

A significant incidence of anemia was noted in children who drank tea regularly (36.2%) and those from low-income families (31.9%). Based on the analysis residence, malaria, MUAC, tea drinking and family income were identified as factors to be tested for association with anemia in multivariate analysis by considering P-value less than

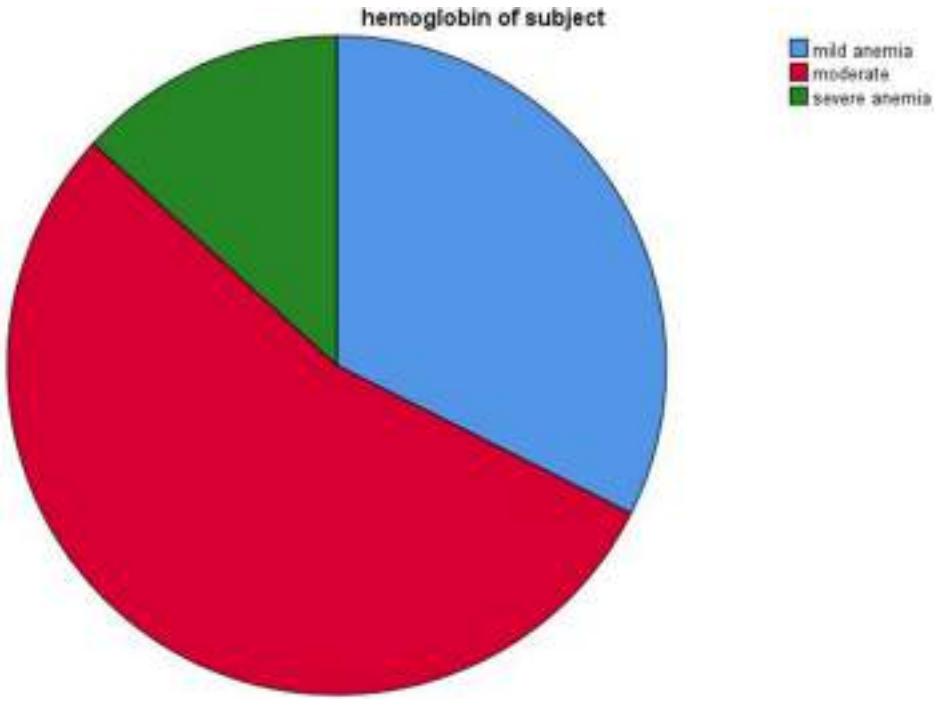

Figure 1 Magnitude and severity pattern of anemia among hospitalized children of 6–59 months age at Jimma Medical Center, Southwest Ethiopia from June 15 to October 15, 2022.

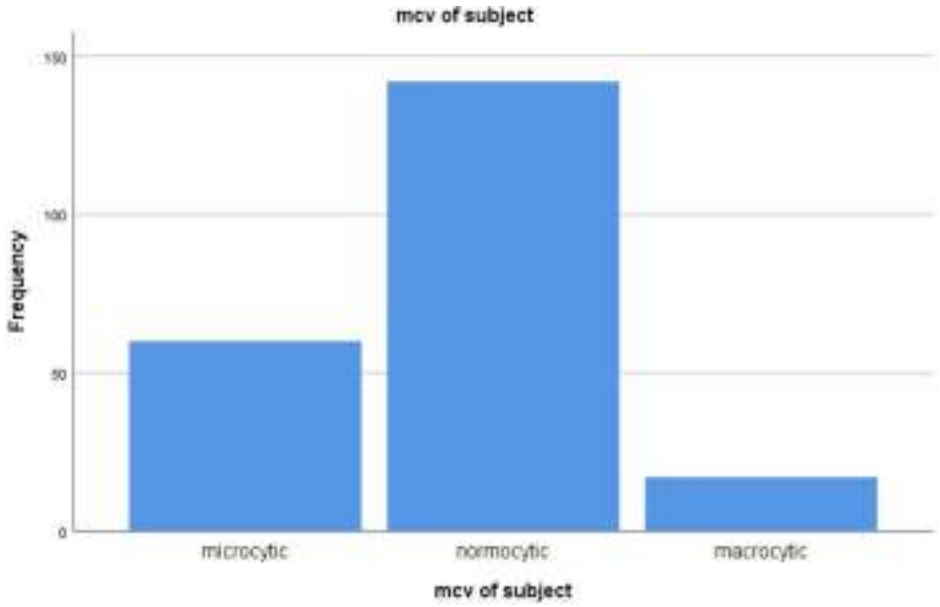

Figure 2 Morphological classification of anemia among hospitalized children of 6–59 months age at Jimma Medical Center, Southwest Ethiopia from June 15 to October 15, 2022.

or equal to 0.25 in bivariable logistic regression. However, in multivariate binary logistic regression analysis, malaria, MUAC, and family income were only still substantially linked with anemia in children (ages 6–59 months) (Table- 4).

Discussion

One of the biggest issues with public health in poor nations is anemia. According to estimates, there are 293.1 million anemic children under the age of 5 years worldwide, with 28.5% of these children residing in sub-Saharan Africa (SSA). It is widely acknowledged as a major public health issue with a prevalence of up to 67%, or 83.5 million children in sub-Saharan Africa.[20]

The present study showed that the overall prevalence of anemia among hospitalized 6–59 months old children was 57.2%. Our findings indicated a serious public health issue in the research area, in accordance with the WHO's recommendation.[21] Numerous studies with comparable results have been published from many nations, including Brazil (56.6%)[22], India (60.55%),[23] Ghana (55.0%), and Gondar, Ethiopia (54.1%).[21]

Nevertheless, the present study's findings were less than those of a study in India, 72.79%,[24] in Tanzania 77.2%,[25] and in Southern Tanzania 83.17%.[26] The disparity may result from variations in the study participants' geographic locations, sociodemographic traits, or the socioeconomic standing of the parents in the communities. This may also be due to having vegetarian diets and presence of different types of anemia in those countries.

The study area's outcome was greater than a study done in Brazil 32.8%,[27] in South Lebanon 33.2%,[28] in Uganda 46.6%,[29] in Sudan 49.4%,[38] in Ethiopia at Debra Markos 11.9%,[6] at Assela 36.7%,[30] at Hawassa Ethiopia 41.7%[30] and Shanan Gibe Hospital 48.9%.[17] The seasonal variance in research, the unpredictability of an automated analyzer, regional variations, and societal disparities in lifestyle could all be contributing factors to the disparity in the prevalence of anemia.

According to the study's anemia severity pattern, 18.81%, 30.82%, and 7.57% of participants had mild, moderate, or severe anemia, respectively. The extent of severe anemia was in line with research conducted in Uganda (11.9%) and in Assela (6.2%),[31] and research from Shanan Gibe Hospital in Ethiopia 8.2%.[17]

However it is lower than a study conducted in Ghana 24%,[32] in Tanzania 27.7%,[25] in Southern Tanzania 46.03%[33] in Ethiopia at Gondar 20.9%,[34] at Hawassa, Ethiopia 16.1%,[30] The reduction in severity observed in this study could be attributed to various reasons such as the implementation of current dietary therapies, public health initiatives, and the ease of access to health information provided by health extension workers.[6]

The present study result was higher than a study done in South Lebanon with 2 cases of severe anemia,[35] the variability of this result may be because of variation of knowledge among different regions' society in a country or due to sample size variation.

According to the study, the majority of our participants with anemia had a normocytic normochromic blood picture 36.5%, this could be brought on by medication use, long-term illnesses, or inflammation-related anemia, which results in a slightly reduced erythrocyte survival rate (increased destruction). Hyperemia, erythropoiesis inhibited by iron due to cytokine-stimulated hepcidin elevation, Hepcidin excretion from the kidneys and the direct actions of cytokines on the bone marrow can both suppress erythropoiesis. Inflammation can also have varying effects on the production of erythropoietin.[36]

But the second kind of anemia in this investigation was microcytic-hypochromic anemia. The high percentage of microcytic-hypochromic anemia is thought to be caused by iron depletion (hemoglobin levels are normal, but the body has a small amount of stored iron that will soon run out), decreased iron in diet, poor gut absorption of iron, acute and chronic blood loss, and increased demand for iron in children for rapid growth.[35] Our findings disagreed with studies done in Uganda in which microcytic hypochromic anemia was 65.4%,[29] in Ghana microcytic hypochromic anemia was 52%,[32] in Tanzania, microcytic hypochromic anemia accounted for 37.5% of the anemia cases in children[25], and in Kenya on morphological patterns of anemia the microcytic pattern was the most common, representing 42.3%.[37] This variation may be closely related to socioeconomic differences and dietary variation.

In the present study anemia was high among children whose age is < 1 year (6–11 months), with prevalence of 15.6% among anemic children. This could be caused by diets deficient in bioavailable iron, low maternal iron reserve during pregnancy, and high iron demands linked to erythropoiesis and rapid growth rate.[35] Moreover, the younger children are more susceptible to infections and diseases which inhibit their iron absorption.

Furthermore, the prevalence of anemia was higher in male children (33.43%). This could be attributed to the fact that preschool-aged males develop quicker than females, which raises their iron needs beyond what can be satisfied by diet.[38]

However, additional research is needed to fully comprehend this aspect. If the body does not receive the proper compensation for this physiological situation, iron deficiency leads to IDA anemia. This finding also shown that children with urban residence have high chance of being anemic with 30.03%, but this might be because many study participants was from an urban area.

The finding additionally shows high magnitude of anemia among children who had habit of tea drinking 36.26%, this might be because tea interferes with iron absorption and can lead to iron deficiency when consumed in large quantities. The process involves the naturally occurring tea chemicals tannins and oxalate, which bind iron—specifically, non-heme iron—found in plant foods such as beans, peas, leafy green vegetables, and nuts.[20]

In this study, factors associated with children (aged 6–59 months) having anemia were malaria infection (AOR = 1.15, 95% CI: 0.017, 0.781, P = 0.001), MUAC (AOR = 2.046, 95% CI: 0.306, 1.366, P = 0.045) and family income (AOR = 2.6, 95% CI: 0.475, 0.894, P = 0.043). Malaria infection was significantly associated with children (aged 6–59 months) having anemia which is supported by a study reported in Uganda,[29] Tanzania and Sudan. Children with malaria infection were ½ times more likely to be anemic as compared with children without malaria infection. Malaria is known to cause anemia through different mechanisms that include a decrease in erythrocytes production or an increase in erythrocytes loss or both. (Malaria is caused by an intra-erythrocytic parasite so there is obligatory destruction of red cells containing parasites at schizont-rupture. But the more important contributor is the accelerated destruction of non-parasitized red cells that parallels disease severity.)[23]

MUAC was also significantly associated with study subject (6–59 month children) anemia which was supported by a study reported in south Lebanon.[35] It was indicated that children with severe malnutrition were 2 times more likely anemic than children with normal nutrition. This might be due to their rapid body growth and their high RBCs expansion, children below 5 years of age have increased iron needs, as a result they are more susceptible to develop anemia.[35]

Additionally, family income was also significantly associated with study subject (aged 6–59 months children) anemia which is supported by a study reported in Uganda,[29] Tanzania[25] and Sudan.[39] It shown that children with a low family income are two and a half times more likely to be anemic then high family income children. A possible reason for the association might be due to families with low income being less likely to buy nutrient-rich foods (like iron, vitamins etc.), secure food availability, and not being able to afford health-care service during illness for their children. Therefore, it is necessary to engage women in income-generating activities so that their children have better health care and supplementary food.

Conclusion

In general, anemia among 6–59 months old children was a major public health problem in the study area and these results indicate that anemia is still an important public health problem even though interventions have been made. The severity of anemia among anemic clients shows moderate anemia was the highest and morphological classification of anemia indicates that most of anemic blood picture was normocytic-normochromic. Malaria parasitemia, nutritional status of child (using MUAC) and family annual income were the factors significantly associated with anemia.

Abbreviations

ANC, Antenatal care; AOR, adjusted odds ratio; CBC, complete blood count; CD, chronic disease; CHr, reticulocyte hemoglobin content; CI, confidence interval; COR, crude odds ratio; CSA, central statistics agency; EDHS, Ethiopia demographic health survey; EDTA, Ethylenediaminetetra acetic acid; HFIAS*, Household food insecurity access scale; Hgb, hemoglobin; IRB, institutional review board; JMC, Jimma Medical Center; MCV, mean corpuscular volume; MUAC, mid-upper arm circumference; NACS, Nutrition Assessment; Counseling, and Support; RBC, red blood cell; SAM, severe acute malnutrition; SCD, sickle cell disease; SOP, standard operation procedure; SSA, sub-Saharan Africa; UTI, urinary tract infection; WHO, World Health Organization.

Data and Materials

The necessary data analyzed during the current study are available from the corresponding author when requested.

Ethical Considerations

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/837, permission to conduct the study was obtained from the Head of School of Medical Laboratory Science and chief clinical director of the JMC. A support letter from Jimma University Health Science Research Coordinating Office was written to JMC. After discussing the research aims to each participant's mother, parents, or guardians, they was asked to sign an informed written consent and assent form, and those who were willing to participate were included in the study. Participation was fully voluntarily, refusal at any time during data collection was permitted. Confidentiality was kept. Any abnormal test results of participants were communicated to their attending physician immediately to make proper management and treatment.

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Disclosure

The authors have declared that they have no existing competing interests in this work.

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ORIGINAL RESEARCH

Presentation and Outcome of Patients with Multiple Myeloma (MM), Single Centre Experience from Windsor Essex Regional Cancer Centre

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Introduction: Outcomes for patients with multiple myeloma has significantly improved through the years. This is mainly related to the use of novel agents.

Methods: This is a retrospective study that reviewed presentation and outcome of 139 patients with multiple myeloma at the Windsor Essex Regional Cancer Centre from Jan. 1, 2015 to Dec. 31, 2019. Median age was 71 years and most patients had higher risk disease (65.5% either R ISS stage II or III). 30% had high risk FISH for myeloma including del.17P, t (4:14), t (14:16) and Gain (1q21). In terms of presentation, 38.8% had anemia (hemoglobin <100g/L), 18.7% had hypercalcemia, 74.1% had skeletal lytic lesions, 38.8% had pathologic fracture and 17.3% had plasmacytoma.

Results: Almost all (92%) of the patients were treated using at least one novel agent (proteasome inhibitor or immunomodulators [ImiDs]). Cyclophosphamide, bortezomib, and dexamethasone (CyBorD) was the most used treatment regimen (48.9%) followed by bortezomib, melphalan and prednisone (BMP) at 28.8% and lenalidomide, dexamethasone (LenDex) at 14.4%. With respect to response to therapy, 51.8% had at least Very good partial response (VGPR), while 9.4% had progressive disease. 33% had autologous stem cell transplant. After a median follow up of 2.4 years, median overall survival was 3.7 years. 2 years overall survival and relapse-free survival were 70% and 83%, respectively.

Discussion: Our study showed comparable outcome for patients with multiple myeloma despite older age and higher risk disease. Outcome is expected to improve with the introduction of more novel agents.

Keywords: multiple myeloma, proteasome inhibitors, immunomodulators

Introduction

Multiple myeloma was responsible for 1600 deaths in Canada in 2020 with an annual incidence of 3400 cases (https://www.cancer.ca/statistics). Although there are significant advances in its treatment, multiple myeloma is still an incurable disease. The discovery of novel therapeutic agents including proteasome inhibitors and immunomodulators made a huge improvement in the outcome of patients with multiple myeloma.[1]

The newly utilized combination of monoclonal antibodies and dual novel agents has resulted in a dramatic improvement in response rate, minimal residual disease (MRD) negativity and survival.[2,3] However, access to newer anti-myeloma drugs is variable among different countries as well as provinces. This is likely in part responsible for the differences in patient outcomes among different institutions.

In this retrospective chart review, data on demographics, presentation and outcome of patients with multiple myeloma treated at the Windsor Essex Regional Cancer Centre are described. Outcome metrics including median overall survival and relapse-free survival were compared with the outcomes from local as well as international institutions.

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Methods

The study is a retrospective chart review on 139 patients diagnosed and treated for multiple myeloma from Jan. 1, 2015 to Dec. 31, 2019 at the Windsor Essex Regional Cancer Centre. Information on demographics, presentation, treatment and outcome was collected.

The Revised International Staging system (R ISS) was included whenever information was available using the IMWG definition. Patients with high-risk FISH were defined as having one of the following: del.17p, t (4; 14), t (14; 16) and Gain (1q21). Response rates were classified as complete remission (CR), Very good partial response (VGPR), stable disease and progressive disease based on the IMWG criteria.[4,5]

Continuous variables are presented as median (interquartile range [IQR]) and categorical variables are reported as frequency (percentage). Time to all-cause mortality and relapse were assessed graphically using Kaplan-Meier curves. Median survival time was determined using the time where the Kaplan-Meier survival curve crossed the 50% threshold. Characteristics associated with death and relapse were assessed using hazard ratios obtained from univariable Cox proportional hazards models. Two-sided p-values <0.05 were considered statistically significant. All analyses were run using R version 4.0.2.

Results

A total of 139 patients with multiple myeloma were treated at the Windsor Essex Regional Cancer Centre from Jan. 1, 2015 to Dec. 31, 2019.

Median age was 71 years, ranging from 63–78 years, with 52% male. Most patients had higher risk disease (65.5% either R ISS stage II or III). 30% had high risk FISH for myeloma including del.17P, t (4:14), t (14:16) and Gain (1q21). In terms of presentation, close to half (47%) had moderate to severe renal impairment (eGFR < 60mL/min/1.73m^2), 38.8% had anemia (hemoglobin <100g/L), 18.7% had hypercalcemia, 74.1% had skeletal lytic lesions, 38.8% had pathologic fracture and 17.3% had concurrent plasmacytoma (Table 1).

Table 1 Baseline Characteristics of Patients with Multiple Myeloma

	Overall (N=139)
Age (Years)	
Median (IQR)	71 (63, 78)
Sex	
Male	72 (51.8%)
Female	67 (48.2%)
Revised International Staging System (R-ISS)	
I	8 (5.8%)
II	66 (47.5%)
III	25 (18.0%)
N/A	40 (28.8%)
Glomerular filtration rate (mL/min/1.73m²)	
>90	26 (18.7%)
60–90	46 (33.1%)

(*Continued*)

Table 1 (Continued).

	Overall (N=139)
30–59	38 (27.3%)
15–29	14 (10.1%)
<15	13 (9.4%)
Missing	2 (1.4%)
Ionized calcium (mmol/L)	
Normal (<=1.29)	107 (77.0%)
Elevated (>1.29)	26 (18.7%)
Missing	6 (4.3%)
Hemoglobin (g/L)	
≥100	85 (61.2%)
<100	54 (38.8%)
Lactate dehydrogenase (LDH) (U/L)	
Normal (<234)	110 (79.1%)
Elevated (≥234)	25 (18.0%)
Missing	4 (2.9%)
Skeletal lytic lesions	
Yes	103 (74.1%)
No	35 (25.2%)
Missing	1 (0.7%)
Pathologic fracture	
Yes	54 (38.8%)
No	84 (60.4%)
Missing	1 (0.7%)
Plasmacytoma	
Yes	24 (17.3%)
No	114 (82.0%)
Missing	1 (0.7%)
Myeloma Fluorescence In Situ Hybridization	
Negative	45 (32.4%)
High Risk	43 (30.9%)
Standard Risk	3 (2.2%)
N/A	48 (34.5%)

Almost all (92%) of the patients were treated using at least one novel agent (proteasome inhibitor or ImiDs). Cyclophosphamide, bortezomib, and dexamethasone (CyBorD) was the most commonly used treatment regimen (48.9%) followed by bortezomib, melphalan and prednisone (BMP) at 28.8% and lenalidomide, dexamethasone (LenDex) at 14.4%. Only five patients received melphalan-dexamethasone. 33% had autologous stem cell transplant and 31% were on maintenance therapy for a median of 10 months (Table 2).

With respect to response to therapy, 51.8% had at least Very good partial response (VGPR) of which 12.2% had Complete response (CR). 9.4% had progressive disease. The rest include partial response, stable disease and missing (Table 2).

Table 2 Treatment and Outcome of Patients with Multiple Myeloma

	Overall (N=139)
Initial chemotherapy regimen	
Lenalidomide-Dexamethasone	20 (14.4%)
Bortezomib-Melphalan-Prednisone	40 (28.8%)
Cyclophosphamide-Bortezomib-Dexamethasone	68 (48.9%)
Melphalan-Dexamethasone	5 (3.6%)
Missing	6 (4.3%)
Number of chemotherapy cycles	
Median (IQR)	5 (4, 7)
Missing	1 (0.7%)
Best Response	
Complete response	17 (12.2%)
Very good partial response	55 (39.8%)
Partial response	31 (22.3%)
Stable disease	12 (8.6%)
Progressive disease	13 (9.4%)
Missing	11 (7.9%)
Toxicity requiring dose reduction/stop	
Yes	28 (20.1%)
No	94 (67.6%)
N/A	10 (7.2%)
Missing	7 (5.0%)
Toxicity type	
Peripheral neuropathy	3 (2.2%)
Diarrhea	3 (2.2%)
Fatigue	1 (0.7%)

(*Continued*)

Table 2 (Continued).

	Overall (N=139)
Cytopenia	4 (2.9%)
Other	15 (10.8%)
Multiple types	3 (2.2%)
N/A	94 (67.6%)
Missing	16 (11.5%)
Palliative radiation	
Yes	64 (46.0%)
No	73 (52.5%)
Missing	2 (1.4%)
First autologous stem cell transplant	
Yes	46 (33.1%)
No	90 (64.7%)
Missing	3 (2.2%)
Tandem stem cell transplant	
Yes	3 (2.2%)
No	134 (96.4%)
Missing	2 (1.4%)
Maintenance therapy	
Yes	44 (31.7%)
No	92 (66.2%)
Missing	3 (2.2%)
Maintenance chemotherapy regimen	
Lenalidomide	35 (25.2%)
Bortezomib	3 (2.2%)
Ixazomib	1 (0.7%)
Lenalidomide-Ixazomib	4 (2.9%)
Lenalidomide-Dexamethasone	1 (0.7%)
N/A	95 (68.3%)
Duration of maintenance therapy (months)	
Median (IQR)	10 (5, 20)
Missing	95 (68.3%)

(*Continued*)

Table 2 (Continued).

	Overall (N=139)
Palliative care referral	
Yes	41 (29.5%)
No	97 (69.8%)
Missing	1 (0.7%)

Patients were followed for a median of 2.4 years with 61 deaths and 31 relapse events. Median overall survival was 3.7 years. Two years overall survival and relapse-free survival were 70% and 83%, respectively. One year survival probability was 80% (Figures 1 and 2).

Cox regression showed hypercalcemia as the only variable significantly associated with increased risk of relapse (HR 2.76, 95% CI 1.28–5.97, P value 0.009). On the other hand, older age (HR 1.03 per year, 95% CI 1.05–1.05, P value 0.02), response inferior to VGPR (stable disease/partial response) (HR 2.87, 95% CI 1.57–5.27, P<0.0001) and progressive disease (HR 10.25, 95% CI 4.99–21.06, P<0.0001) were significantly associated with risk of death. In addition, having autologous stem cell transplant (HR 0.18, 95% CI 0.08–0.38, P value 0.0001) and being on maintenance therapy (HR 0.25, 95% CI 0.12–0.51, P < 0.0001) were significantly associated with reduced risk of dying (Tables 3 and 4).

Discussion

Despite its limitations including small sample size and retrospective design with missing variables, the study revealed important information on the presentation, treatment and outcome of patients with multiple myeloma at the Windsor

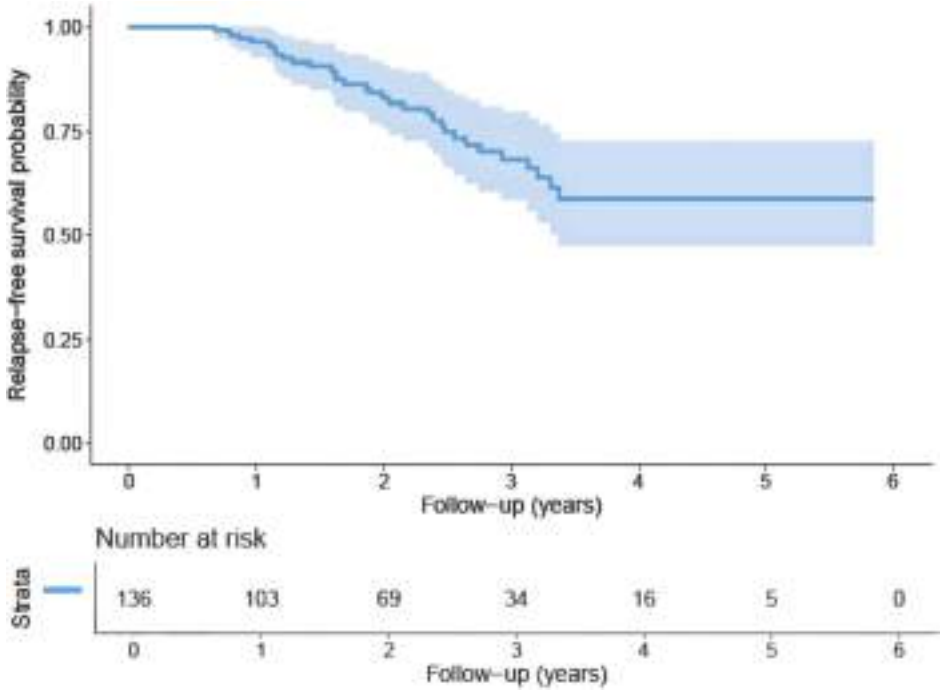

Figure 1 Shows The Kaplan-Meier curve for patients' relapse-free survival in years.

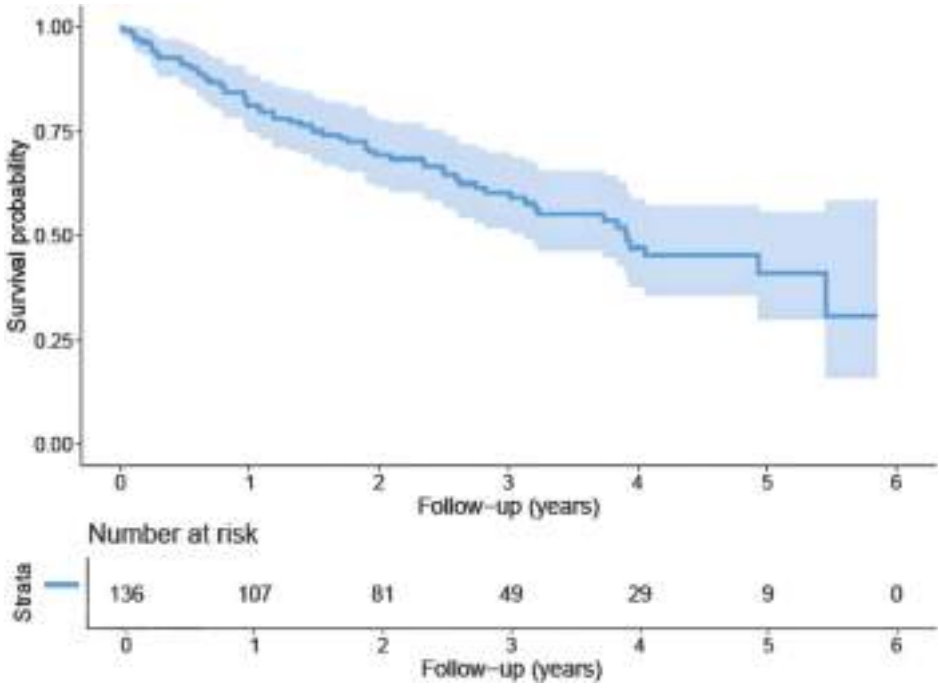

Figure 2 Shows The Kaplan-Meier curve for patients' overall survival in years.

Essex Regional Cancer Centre. Outcome of patients in this study is comparable with other centres in Canada as well as patients internationally treated with similar regimen. However, the outcome is inferior to those centres that used dual novel agents with or without monoclonal antibody.[6,7]

A retrospective study from Alberta multiple myeloma registry on 147 patients with median age of 73 years showed median overall survival of 3.3 years for those on CyBorD regimen, which is comparable to our study (ASH poster 2020). Another study from the Swedish myeloma registry on 4904 patients from 2008 to 2015 showed median overall survival

Table 3 Hazard Ratios for Baseline Characteristics and the Outcome of Relapse

Variable	Value	Unadjusted			
		Hazard Ratio	95% CI		P-value
Age, per year		0.99	0.96	1.02	0.48
Sex					
	Female	0.73	0.36	1.49	0.39
	Male	1.00	(referent)		
R-ISS					
	I or II	1.00	(referent)		
	III	2.20	0.88	5.45	0.09
Ionized calcium					
	Normal (<=1.29)	1.00	(referent)		
	Elevated (>1.29)	2.76	1.28	5.97	0.0099

(*Continued*)

Table 3 (Continued).

Variable	Value	Unadjusted			
		Hazard Ratio	95% CI		P-value
Glomerular filtration rate					
	≥60	1.00	(referent)		
	<60	1.62	0.78	3.33	0.19
Pathologic fracture					
	No	1.00	(referent)		
	Yes	1.50	0.74	3.06	0.26
Hemoglobin					
	<100	1.37	0.66	2.82	0.40
	≥100	1.00	(referent)		
Best response					
	Complete or very good partial response	1.00	(referent)		0.90
	Partial response or stable disease	0.94	0.42	2.13	
	Progressive disease	1.72	0.40	7.39	
Autologous stem cell transplant					
	No	1.00	(referent)		
	Yes	0.71	0.34	1.46	0.35
Maintenance therapy					
	No	1.00	(referent)		
	Yes	0.80	0.38	1.66	0.54

of 3.4 years for those patients older than 65 years of age which is again comparable with our outcome. However median overall survival was much higher at 7.7 years for those patients 65 years or younger suggesting the strong impact of age on survival.[6] Another retrospective study on 1000 patients from the Emory myeloma database from January 2007 to August 2016 showed much higher median overall survival of 10.5 years. However, these patients were treated with a regimen that has dual novel agents [RVD: Revlimid (ImiD) and velcade (proteasome inhibitor)]. In the same study response of VGPR or better was also much higher at 89% compared with 51% in our study.[7] This underscores the importance of using multiple novel agents to get a deeper response in the initial treatment of multiple myeloma. With the discovery of more and more novel therapies for the treatment of multiple myeloma, access to these drugs will be critical.

This is clearly demonstrated in the dramatic response seen with the combination of monoclonal antibody, daratumumab, and dual novel agents (ImiDs or proteasome inhibitors) in the frontline treatment of patients with multiple myeloma with a complete response rate of up to 79% and minimal residual disease (MRD) negativity of 14–24%.[2,3]

The most widely used, currently funded, first-line treatment regimen for transplant eligible patients with multiple myeloma in Windsor Essex is CyBorD. Whereas BMP and recently LenDex are funded and commonly used as first-line therapy for those who are ineligible for autologous stem cell transplant. (Systemic Treatment - Quality Based Procedure, Cancer Care Ontario; https://www.cancercareontario.ca/en/cancer-treatments/chemotherapy/funding-reimbursement/systemic-treatment-quality-based-procedure).

Table 4 Hazard Ratios for Baseline Characteristics and the Outcome of All-Cause Mortality

Variable	Value	Unadjusted			
		Hazard Ratio	95% CI		P-value
Age, per year		1.03	1.01	1.05	0.02
Sex					
	Female	0.89	0.54	1.48	0.65
	Male	1.00	(referent)		
R-ISS					
	I or II	1.00	(referent)		
	III	0.98	0.43	2.27	0.97
Ionized calcium					
	Normal (<=1.29)	1.00	(referent)		
	Elevated (>1.29)	1.53	0.85	2.78	0.16
Glomerular filtration rate					
	≥60	1.00	(referent)		
	<60	1.41	0.84	2.37	0.19
Pathologic fracture					
	No	1.00	(referent)		
	Yes	0.71	0.42	1.22	0.22
Hemoglobin					
	<100	1.33	0.80	2.22	0.27
	≥100	1.00	(referent)		
Best response					
	Complete or very good partial response	1.00	(referent)		<0.0001
	Partial response or stable disease	2.87	1.57	5.27	
	Progressive disease	10.25	4.99	21.06	
Autologous stem cell transplant					
	No	1.00	(referent)		
	Yes	0.18	0.08	0.38	<0.0001
Maintenance therapy					
	No	1.00	(referent)		
	Yes	0.25	0.12	0.51	<0.0001
Relapse (time-varying covariate)					
	No	1.00	(referent)		
	Yes	2.03	0.96	4.29	0.06

Although access to combination of novel agents is slowly improving, the data are compelling to incorporate multiple novel agents and monoclonal antibodies in the front-line setting. This will result in deeper response and higher rate of MRD negativity which in turn will have positive impacts on survival.[2,3] Regulatory agents will need to consider this emerging scientific evidence and cost-benefit implications for society in deciding drug access, particularly in multiple myeloma.

Hypercalcemia was the only variable significantly associated with higher risk of relapse (HR 2.76, 95% CI 1.28–5.97, P 0.009) but not death in our study. Other studies on patients with symptomatic MM showed inferior survival in those patients with hypercalcemia.[8]

In our study, older age and poor response to therapy, particularly progressive disease (HR 10.2, 95% CI 4.99–21, P< 0.0001) were significantly associated with higher risk of death. On the other hand, those with autologous stem cell transplant had significantly lower risk of dying (HR 0.18, 95% CI 0.08–0.38, P < 0.0001). This could be associated with selection bias as patients who had autologous stem cell transplant are generally younger and healthier. Better survival for those on maintenance therapy can also be subjected to similar bias as patients who live longer are more likely to be on maintenance. Nevertheless, survival benefit for autologous stem cell transplant as well as maintenance therapy was demonstrated in several other studies.[4,9–11]

The impact of relapse on survival as a time-dependent variable was close to statistical significance but not quite, unlike other studies which showed early relapse as a risk for poor survival.[12]

Finally, despite the continuous effort of the scientific community in finding new and novel treatment options for multiple myeloma, studies still suggest that the outcome of patients with 17p deletion and t (4; 14) has not improved dramatically.[13,14] This underscores the need for continued bench to bedside collaboration between scientists and clinicians for continued breakthrough in the fight against myeloma.

In conclusion, our study showed comparable outcomes for patients with multiple myeloma treated with similar treatment regimen in other centres. However, improved patient outcomes were observed with newer regimens that incorporate monoclonal antibodies and dual novel agents. As more and more novel drugs are introduced to the armament against the disease, it is expected that access to these drugs will affect outcomes. We recommend a prospective myeloma registry to acquire more comprehensive information as well as improved access to combination of novel agents and monoclonal antibodies to improve the outcome of our patients with multiple myeloma.

Ethical/Copyright

The study was cleared by the Windsor Regional Hospital (WRH) Ethics Review Board and supported by the Schulich-Windsor Opportunities for Research Excellence Program (SWORP). The study was exempt from informed consent as it was judged to not involve more than minimal risk as approved by the ethics review board of WRH. Maximum precaution was used to protect patient confidential data. The study was conducted in compliance with the Declaration of Helsinki.

Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

A Post-Authorization Safety Surveillance Study to Report Clinical Experience with Purified Factor IX Concentrate in Pediatric Patients with Hemophilia B

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Introduction: Purified factor IX (FIX) concentrate (IMMUNINE®, Takeda Manufacturing Austria AG, Vienna, Austria) is indicated for the treatment and prophylaxis of bleeding episodes in patients with congenital hemophilia B. Data on the use of purified FIX concentrate in patients ≤6 years old with congenital hemophilia B are limited.

Aim: Document real-world clinical experience with purified FIX concentrate in routine practice for pediatric patients with hemophilia B.

Methods: This prospective post-authorization safety surveillance study enrolled patients ≤6 years old with moderate or severe hemophilia B (baseline FIX ≤5%) who were prescribed purified FIX concentrate, as determined by the treating physician. The planned observation period for each patient was either 12 months or ≥50 exposure days, whichever occurred first. The primary endpoints were the occurrence of treatment-related adverse events (AEs) and serious AEs (SAEs), and inhibitor development.

Results: Thirteen male patients (mean ± standard deviation age, 3.80 ± 1.76 years) enrolled and received ≥1 treatment with purified FIX concentrate. Thirty-two AEs were reported in 6 patients; 4 were SAEs. No AEs were considered related to purified FIX concentrate. No patients developed inhibitory antibodies. Inhibitor testing was not conducted in 2 patients. Eighteen bleeding episodes were treated with purified FIX concentrate in 6 patients. Hemostatic efficacy was rated as either "excellent" or "good" in all patients with an available rating.

Conclusion: No treatment-related AEs were reported, and purified FIX concentrate was shown to be effective in treating and preventing bleeding episodes in pediatric patients ≤6 years old with hemophilia B.

Keywords: factor IX, hemophilia B, pediatrics, post-marketing product surveillance, surgery

Introduction

Congenital hemophilia B is a rare X-linked bleeding disorder caused by either a deficiency or absence of coagulation factor IX (FIX).[1] The severity of hemophilia B is classified according to the level of residual FIX, with severe hemophilia B defined as a FIX level <1% and moderate hemophilia B defined as a FIX level 1–5%.[1,2] Hemophilia B is characterized by bleeding episodes, particularly into large joints that can lead to painful and debilitating hemophilic arthropathy.[1]

The main treatment approach for patients with hemophilia B is replacement therapy with either plasma-derived or recombinant FIX concentrates.[2] FIX concentrates can be further classified as either pure or containing factors II, VII, IX, and X, also known as prothrombin complex concentrates.[2] The 2020 World Federation of Hemophilia (WFH) guidelines for the management of hemophilia recommend the use of pure FIX concentrates over prothrombin complex concentrates for the treatment of patients with hemophilia B, as pure FIX concentrates are associated with a reduced risk of thrombosis and disseminated intravascular coagulation compared with prothrombin complex concentrates.[2] For patients

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with hemophilia B who are undergoing surgery, these guidelines also recommend the use of pure FIX concentrates rather than prothrombin complex concentrates.[2]

There are many plasma-derived and recombinant FIX concentrates available for the management of patients with hemophilia B, including extended half-life recombinant FIX concentrates which were developed to reduce the treatment burden associated with standard half-life products.[2,3] Replacement therapies can be administered by either bolus injections or continuous infusions as an on-demand therapy for the treatment of bleeding episodes, or as prophylaxis for the prevention of bleeding episodes.[3–5] These treatments can also be used for the perioperative management of patients who are undergoing surgery or invasive procedures.[3] Studies have shown FIX concentrate prophylaxis to be associated with lower bleeding rates compared with on-demand treatment in patients with hemophilia B.[6–9] Individualized prophylaxis is recommended by the 2020 WFH guidelines for the treatment of patients with hemophilia B with a severe phenotype, including patients with moderate hemophilia B who have a severe phenotype.[2] For pediatric patients with severe hemophilia B, the WFH guidelines recommend the early initiation of prophylaxis, ideally before 3 years of age and prior to the onset of joint disease, with either standard or extended half-life FIX concentrates or other hemostatic agents.[2]

FIX concentrate (IMMUNINE®, Takeda Manufacturing Austria AG, Vienna, Austria) is purified from human plasma and contains only traces (≤0.02 IU) of factors II, VII, and X.[10] FIX concentrate has also been shown to contain low levels of activated FIX, a product-related impurity in FIX products that has been associated with an increased risk of thrombogenicity.[4,5] FIX concentrate is indicated for the treatment and prophylaxis of bleeding episodes in patients with congenital hemophilia B.[10,11] However, indications may vary by country. Data on the use of purified FIX concentrate in patients ≤6 years old with hemophilia B are limited.[11] Real-world studies have become increasingly significant for demonstrating treatment effectiveness and safety in routine clinical practice.[12] This post-authorization safety surveillance (PASS) study was designed to document real-world clinical experience with purified FIX concentrate (IMMUNINE®) in routine practice for pediatric patients ≤6 years old with hemophilia B.

Methods

Study Design

This was a prospective, uncontrolled, open-label PASS study involving 14 study sites in 6 countries (Czech Republic, Germany, the Netherlands, Poland, Serbia, and Ukraine). The study surveillance period was from November 2009 to January 2014. The product formulation was the same across all six countries.

The study followed a cohort design and did not make any stipulations on treatment or observation schedule. Prophylaxis and on-demand dosing regimens were determined at the discretion of the treating physician, in accordance with prescribing information. The planned observation period for each patient was ~12 months or ≥50 exposure days (EDs), whichever occurred first (Supplementary Figure 1). A screening visit was required prior to enrollment and was scheduled to coincide with a routine visit to a hemophilia treatment center. A termination visit was planned to coincide with a routine visit at the end of the observation period. No stipulations were made on clinical or laboratory testing beyond what was required to determine patient eligibility.

The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice E6, Title 21 of the US Code of Federal Regulations, the European Clinical Trial Directive, and applicable national and local regulatory requirements, including European regulations pertaining to PASS studies. This study was also conducted in accordance with the Declaration of Helsinki. Approval from Institutional Review Boards/Independent Ethics Committees, listed in Supporting Information, was obtained for the study protocol and informed consent form. All patients or their legally authorized representative signed an informed consent form prior to entering the study.

Patients

Patients with moderate or severe congenital hemophilia B (baseline FIX ≤5%) and ≤6 years old who had been prescribed purified FIX concentrate (IMMUNINE®) by their treating physician were eligible for inclusion. Patients were eligible regardless of whether or not they had previously received treatment with any FIX concentrates, including purified FIX concentrate (IMMUNINE®). Patients known to have disseminated intravascular coagulation or hyperfibrinolysis were not

eligible for inclusion, as were those with FIX inhibitors or any other known clotting factor deficiency. Patients with a known hypersensitivity to the active substance, or any of its excipients, were also excluded from the study.

Endpoints

The primary endpoints were the incidence of adverse events (AEs) and serious AEs (SAEs) that were judged by the treating physician to be at least possibly related to purified FIX concentrate, and the occurrence of high-titer (>5 Bethesda Units [BU]), low-titer (1–5 BU) and transient inhibitor development during the course of treatment. Secondary endpoints included the hemostatic efficacy of prophylaxis and on-demand treatment as measured by the physicians' overall assessment rating of poor, fair, good, or excellent (Supplementary Table 1), and the number of infusions required to achieve bleed resolution.

Statistical Analysis

Descriptive statistical data analyses were carried out for all safety, hemostatic efficacy, and immunogenicity parameters. All patients who received the study drug at any time during the observation period were included in the safety set. The intent to treat (ITT) set consisted of all enrolled patients who received at least 1 dose of study drug and provided any post-treatment data. The per protocol (PP) set was a subset of patients in the ITT set who had completed either 12 months or 50 EDs.

AEs were coded according to the Medical Dictionary for Drug Regulatory Activities version 17.0. The total number of infusions and dose of study drug required to achieve adequate hemostasis for each bleeding episode were reported. The rate of bleeding episodes was calculated (all bleeds and those secondary to trauma) for all patients. Overall hemostatic efficacy of treatment for all bleeding episodes was established based on individual assessment ratings provided by the treating physician. In general, missing data were not replaced except in calculations for age and body weight.

Results

Patients

In total, 13 patients were enrolled at 9 study sites (Poland, n = 7; Germany, n = 2; the Netherlands, n = 2; Czech Republic, n = 1; Serbia, n = 1). No patients were enrolled from Ukraine. All 13 patients received treatment with purified FIX concentrate and were included in the safety and ITT sets. Nine patients were included in the PP set (Figure 1).

All 13 patients were male, with a mean ± standard deviation (SD) age of 3.80 ± 1.76 years (Table 1). None of the 13 patients had a history of FIX inhibitors. All 13 patients had received treatment with purified FIX concentrate (IMMUNINE®) before enrollment. Six patients had also previously received treatment with another high-purity plasma-derived FIX. Nine patients had >50 EDs to FIX concentrate prior to enrollment.

Safety

In total, 32 AEs were reported in 6 patients, of which 4 were considered to be SAEs (Table 2). The 4 SAEs were reported in 3 patients, of whom 2 patients experienced an accidental head injury and 1 patient experienced 2 cases of sepsis. At the time of the accidental head injury, one patient was receiving 48 IU/kg once weekly prophylaxis. This dosing regimen was not changed following the head injury, although the patient did receive a daily 48 IU/kg dose of purified FIX concentrate for 3 days immediately following this SAE. The second patient who experienced a head injury was receiving 30 IU/kg purified FIX concentrate prophylaxis. Following the head injury, the patient received a single 60 IU/kg dose after which the patient continued to receive 30 IU/kg prophylaxis. The dose of purified FIX concentrate was not changed in response to any of the other SAEs. None of the 32 AEs were considered related to purified FIX concentrate, and all events resolved during the surveillance period. No deaths occurred during the surveillance period.

Immunogenicity

Eleven of the 13 patients in the safety set underwent testing for FIX inhibitors. The Bethesda assay was used to test for FIX inhibitors in 6 patients and the Nijmegen assay was used for 2 patients. Both assays were used to test for FIX inhibitors in 2 patients. None of these 10 patients developed inhibitory antibodies (Supplementary Table 2). One patient

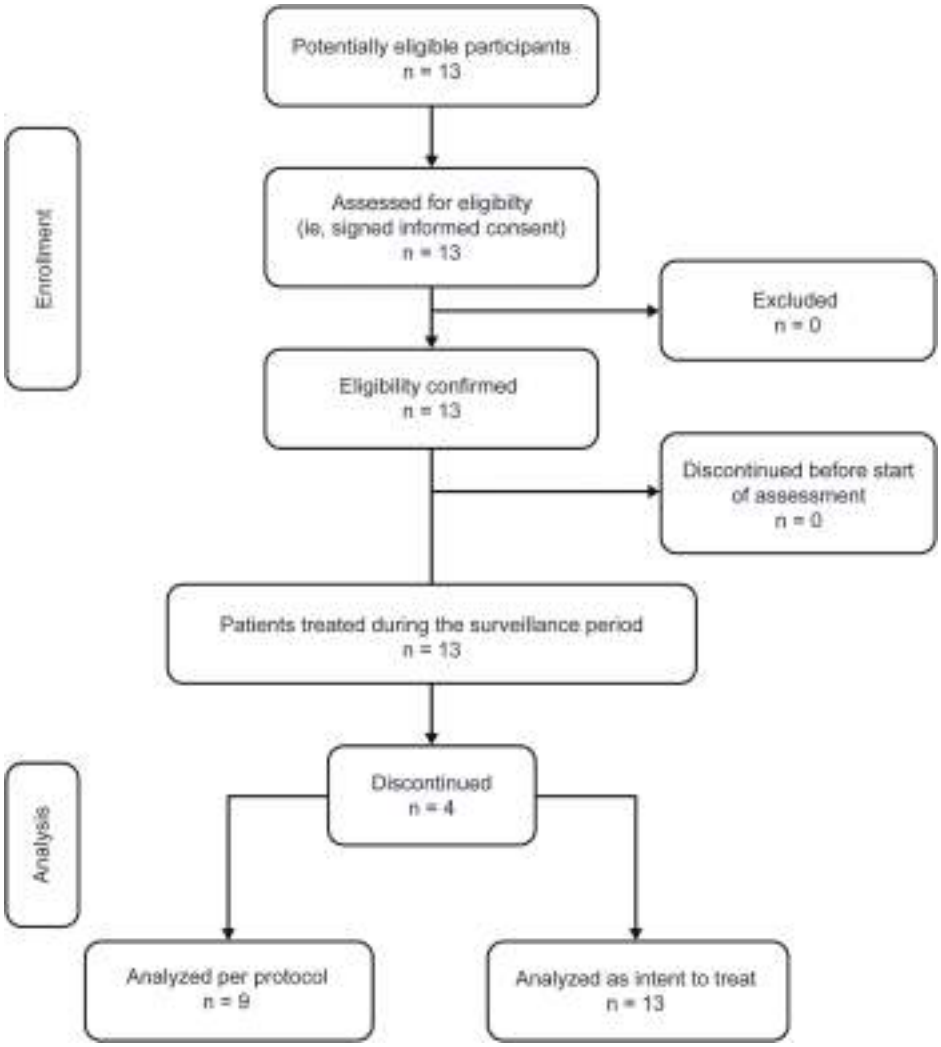

Figure 1 Patient disposition.

underwent testing for FIX inhibitors twice and was reported as negative both times; however, the method used to test for FIX inhibitors was not reported. Inhibitor testing was not conducted in 2 patients. The observation period for 1 patient was 8 months. All other patients were observed for 12 months.

Table 1 Demographics and Baseline Characteristics (ITT Set)

Parameter	**Total, N = 13**
Age, mean ± SD (years)	3.80 ± 1.76
Body weight, mean ± SD (kg)	18.12 ± 4.44
FIX treatment prior to enrollment, n (%) Purified FIX concentrate (IMMUNINE®) Other high-purity plasma-derived FIX	 13 (100) 6 (46.15)

(*Continued*)

Table 1 (Continued).

Parameter	Total, N = 13
Number of FIX EDs prior to enrollment,[a] n (%)	
0 days	0 (0)
1–20 days	2 (15.38)
21–50 days	2 (15.38)
51–150 days	2 (15.38)
>150 days	7 (53.85)
FIX level (%) at enrollment,[b] mean ± SD	1.92 ± 1.57

Notes: [a]No patients reported signs of proteinuria. [b]A value <1% was recorded as 1% in 7 patients.
Abbreviations: ED, exposure day; FIX, factor IX; ITT, intent to treat; SD, standard deviation.

Table 2 Frequency of AEs and SAEs (Safety Set)

AE	Patients, n (%),[a] N = 13	95% CI for Incidence Rates	Events, n
Any AE	6 (46.15)	23.21–70.86	32
Any SAE	3 (23.08)	8.18–50.26	4
Injury, poisoning, and procedural complications	2 (15.38)	4.33–42.24	2
Head injury	2 (15.38)	4.33–42.24	2
Infections and infestations	1 (7.69)	1.37–33.31	2
Sepsis	1 (7.69)	1.37–33.31	2
Any non-serious AE	6 (46.15)	23.21–70.86	28
Infections and infestations	6 (46.15)	23.21–70.86	16
Tonsilitis	2 (15.38)	4.33–42.24	2
Ear infection	1 (7.69)	1.37–33.31	2
Pharyngitis	1 (7.69)	1.37–33.31	2
Acute tonsilitis	1 (7.69)	1.37–33.31	1
Mumps	1 (7.69)	1.37–33.31	1
Nasopharyngitis	1 (7.69)	1.37–33.31	1
Oral herpes	1 (7.69)	1.37–33.31	1
Otitis media acute	1 (7.69)	1.37–33.31	1
Respiratory tract infection	1 (7.69)	1.37–33.31	1
Rhinitis	1 (7.69)	1.37–33.31	1
Urinary tract infection	1 (7.69)	1.37–33.31	1
Viral respiratory tract infection	1 (7.69)	1.37–33.31	1
Viral upper respiratory tract infection	1 (7.69)	1.37–33.31	1
Psychiatric disorders	1 (7.69)	1.37–33.31	2
Anxiety	1 (7.69)	1.37–33.31	2
Respiratory, thoracic, and mediastinal disorders	1 (7.69)	1.37–33.31	1
Cough	1 (7.69)	1.37–33.31	1
Gastrointestinal disorders	1 (7.69)	1.37–33.31	5
Abdominal pain	1 (7.69)	1.37–33.31	2
Constipation	1 (7.69)	1.37–33.31	3
General disorders and administration site conditions	1 (7.69)	1.37–33.31	3
Pyrexia	1 (7.69)	1.37–33.31	3
Injury, poisoning, and procedural complications	1 (7.69)	1.37–33.31	1
Limb injury	1 (7.69)	1.37–33.31	1

Notes: [a]Number of patients with any event; each patient was only counted once in each category.
Abbreviations: AE, adverse event; CI, confidence interval; SAE, serious adverse event.

Bleeding Episodes and Treatment

In the ITT set, 18 bleeding episodes in 6 patients were treated with purified FIX concentrate. Of these, 10 bleeding episodes were classified as traumatic, 7 were classified as spontaneous, and 1 was undetermined. Thirteen bleeding episodes occurred in a joint, 1 occurred in muscle, and 4 were classified as "other". All 18 bleeding episodes were assessed as either minor or moderate in severity. Of the 18 bleeding episodes treated with purified FIX concentrate, 14 episodes in 4 patients were included in the PP set.

Annualized bleeding rates are presented by treatment regimen in Supplementary Table 3 and by cause of bleeding episode in Supplementary Table 4. Table 3 presents the average number of infusions per bleeding episode and the average number of infusions during prophylaxis. The mean ± SD number of infusions per bleeding episode was 1.18 ± 0.57 in the ITT set and 1.03 ± 0.53 in the PP set. The mean ± SD number of infusions administered per year was 91.67 ± 25.15 for patients receiving prophylaxis (n = 11).

Hemostatic Efficacy

In the ITT set, hemostatic efficacy ratings were reported in 7 patients over the surveillance period (Table 4 and Supplementary Table 5). All ratings were either "excellent" or "good". For patient #2, a hemostatic efficacy rating of "good" was given by the

Table 3 Average Number of Infusions

	Per Bleeding Episode[a]		During Prophylaxis, per Year[b]		During Prophylaxis, per Week[c]	
	ITT Set, N = 13	PP Set, N = 9	ITT Set, N = 13	PP Set, N = 9	ITT Set, N = 13	PP Set, N = 9
Patients, n	6	4	11	8	11	8
Mean ± SD	1.18 ± 0.57	1.03 ± 0.53	91.67 ± 25.15	95.87 ± 26.58	1.76 ± 0.48	1.84 ± 0.51
Median (IQR) [range]	1.27 (1.00–1.33) [0.24–2.00]	1.27 (0.72–1.33) [0.24–1.33]	97.68 (63.22–104.36) [53.25–129.26]	103.57 (75.44–113.22) [53.25–129.26]	1.87 (1.21–2.00) [1.02–2.48]	1.98 (1.45–2.17) [1.02–2.48]

Notes: [a]Average number of infusions per bleeding episode: number of bleeding-associated infusions / number of bleeding episodes. [b]Average number of infusions per year: number of infusions under prophylaxis / [(number of study days on prophylaxis) / 365.25]. [c]Average number of infusions per week: number of infusions under prophylaxis / [(number of study days on prophylaxis) / 7].
Abbreviations: IQR, interquartile range; ITT, intent to treat; PP, per protocol; SD, standard deviation.

Table 4 Hemostatic Efficacy Ratings by Physician for Bleeding Episodes Treated with the Study Drug Over the Observation Period

Patient #	Bleeding Episodes, n	Assessment Visit[a]	Rating at Assessment Visit
1	2	Interval	Good
2[b]	1	Termination	Good
3	4	Interval	Excellent
3	1	Termination	Excellent
4	3	Termination	Excellent
5	1	Interval	Good
5	1	Termination	Good
6	2	Interval	Good
6	1	Interval	Good
7[c]	1	Interval	Excellent

Notes: [a]The last rating for a patient is defined as a "termination" assessment; all other ratings are defined as "interval" assessments. [b]Detailed assessment of this bleeding episode was not documented. [c]This patient also received treatment for an additional bleeding episode with a product other than the study drug.

Table 5 Number of EDs to Study Drug and Average Dose per Infusion (ITT Set)

	Prophylaxis, n = 11	Prophylaxis[a] for Surgery, n = 2	On-Demand[a] Treatment, n = 6	Total, N = 13
Number of EDs[b]				
Mean ± SD	73.64 ± 36.97[c]	5.00 ± 4.24	3.67 ± 1.03	64.77 ± 42.87
Median (IQR)	57.00 (46.00–119.00)	5.00 (2.00–8.00)	4.00 (3.00–4.00)	57.00 (48.00–83.00)
[range]	[33–134]	[2–8]	[2–5]	[4–135]
Average dose per infusion (IU)[d]				
Mean ± SD	655.88 ± 183.82	600.00 ± 0	766.67 ± 222.86	683.45 ± 200.94
Median (IQR)	600.00 (600.00–600.00)	600.00 (600.00–600.00)	650.00 (600.00–1050.00)	600.00 (600.00–607.14)
[range]	[600.00–1210.08]	[600.00–600.00]	[600.00–1050.00]	[600.00–1210.08]
Average dose per infusion per kilogram of body weight (IU/kg)[d]				
Mean ± SD	38.29 ± 8.86	42.38 ± 2.92	38.10 ± 12.47	38.10 ± 10.31
Median (IQR)	36.80 (30.00–44.44)	42.38 (40.31–44.44)	38.74 (28.30–49.72)	37.27 (30.00–44.44)
[range]	[28.33–55.60]	[40.31–44.44]	[20.78–52.33]	[20.78–55.60]
Average dose per infusion per kilogram of body weight per year (IU/kg)[b,e]				
Mean ± SD	51.78 ± 24.05[f]	114.60 ± 75.66	42.75 ± 20.94	48.18 ± 26.51
Median (IQR)	56.57 (28.39–68.81)	114.60 (61.10–168.10)	41.25 (22.89–63.72)	39.25 (28.04–66.60)
[range]	[22.00–96.06]	[61.10–168.10]	[21.14–66.23]	[21.14–107.14]

Notes: [a]Some patients received both prophylaxis and on-demand treatment; therefore, the number of patients does not add up to the total (N = 13). [b]Each patient was only counted once in each regimen. [c]For 1 patient receiving prophylaxis, the last documented infusion with the study drug on August 12, 2010 was not taken into account because the patient switched to another product on May 13, 2010. [d]Multiple entries were possible; patients were counted only once in each regimen. [e]Dose per kilogram of body weight per year = average dose per infusion day (IU/kg) / [(last study date − date of enrollment +1) / 365.25]. [f]For 1 patient receiving prophylaxis, only study days until May 12, 2010 were included in the surveillance period because the patient switched to another product. For 1 patient receiving prophylaxis, study days from April 12, 2010 to August 6, 2010 were excluded from the surveillance period because the patient received another product. For 1 patient receiving prophylaxis, only study days until June 23, 2010 were included in the surveillance period because the patient received another product. For 1 patient receiving prophylaxis, only study days until November 8, 2010 were included in the surveillance period because the patient received another product.
Abbreviations: ED, exposure day; IQR, interquartile range; ITT, intent to treat; SD, standard deviation.

treating physician at the termination assessment; however, no details of a bleeding episode were documented in this patient. There were 6 patients in whom no bleeding episodes were reported, and no hemostatic efficacy ratings were provided.

Treatment Regimen

In the ITT set (N = 13), 11 patients received prophylaxis, 6 patients received on-demand treatment, and 2 patients received prophylaxis for surgery. EDs and study drug dose for each regimen are shown in Table 5. The average total dose of study drug per kilogram of body weight per bleeding episode (IU/kg) is presented in Supplementary Table 6. The mean ± SD dose per infusion per kilogram of body weight was 38.29 ± 8.86 IU/kg for patients receiving prophylaxis and 38.10 ± 12.47 IU/kg for patients receiving on-demand treatment.

Discussion

The aim of this PASS study was to document real-world clinical experience with purified FIX concentrate in routine practice for pediatric patients ≤6 years old with hemophilia B. Post-authorisation studies are used to obtain further information on the safety and effectiveness of a therapeutic agent in clinical practice and have been undertaken for other products available for the management of patients with hemophilia.[13–15] In terms of study design, the approach used in this study is different to a safety assessment that was undertaken for another marketed plasma-derived highly purified FIX concentrate (Replenine®; Bio Products Laboratory, Elstree, UK), in which hospital notes were retrospectively reviewed for 114 patients with hemophilia B.[15] The study identified 9 AEs, of which 4 were considered possibly related to the product, and no new FIX inhibitors developed.[15]

All 13 patients who enrolled in this PASS study were included in the safety and ITT sets. There were 9 patients who completed the study in line with the protocol. The mean patient age at enrollment was slightly above the treatment initiation age recommended by the 2020 WFH guidelines for the management of pediatric patients with severe hemophilia B.[2] However, all enrolled patients had previously received treatment with purified FIX concentrate (IMMUNINE®), and some patients had also received previous treatment with another high-purity plasma-derived FIX.

None of the 32 AEs, including 4 SAEs, reported in this study were considered related to purified FIX concentrate, and all events resolved during the surveillance period. None of the 13 patients had a history of FIX inhibitors and there was no evidence of FIX inhibitor development in any of the 11 patients who received treatment with purified FIX concentrate and were assessed for the development of inhibitory antibodies.

Eighteen bleeding episodes in 6 patients were treated with the study drug. The mean ± SD dose per infusion per kilogram of body weight for patients receiving prophylaxis (n = 11) was 38.29 ± 8.86 IU/kg and for patients receiving on-demand treatment (n = 6) was 38.10 ± 12.47 IU/kg. Patients received prophylaxis either once or twice a week. At the time this PASS study was being conducted, the 2013 WFH guidelines made reference to the Malmö protocol (25–40 IU/kg twice per week) and the Utrecht protocol (15–30 IU/kg twice per week) regarding prophylaxis dosing regimens for patients with hemophilia B.[16] For prophylaxis with standard half-life clotting factors in patients with hemophilia B, the 2020 WFH guidelines define high-dose prophylaxis as 40−60 IU FIX/kg twice per week (>4000 IU/kg per year), intermediate-dose prophylaxis as 20−40 IU FIX/kg twice per week (2000–4000 IU/kg per year), and low-dose prophylaxis as 10−15 IU FIX/kg 2 days per week (1000–1500 IU/kg per year).[2]

It should be acknowledged that patient recruitment was challenging, which resulted in a small number of patients enrolled into the study. The difficulties encountered in recruiting patients to this study are likely owing to the rarity of hemophilia B and the fact that this study focuses on a very specific age demographic. In addition, there were 4 patients who did not complete the study according to protocol, which may, in part, have been due to the young age of the patients. Despite a small patient population, this PASS study does provide real-world data on the use of purified FIX concentrate in routine clinical practice for the treatment of pediatric patients ≤6 years old who have hemophilia B.

Conclusions

Over the years, the therapeutic landscape for the management of patients with hemophilia B has expanded beyond plasma-derived replacement therapies to include recombinant FIX concentrates and, subsequently, extended half-life recombinant FIX products.[3,17] Prophylaxis with extended half-life recombinant FIX concentrates has been shown to be effective in the treatment of bleeding episodes and associated with low annualized bleeding rates in patients with hemophilia B.[18–20] The prolonged half-life of these products is also advantageous as they can reduce dosing frequency to once every 7–14 days.[18–20]

For pediatric patients with severe hemophilia B, the 2020 WFH guidelines recommend the early initiation of prophylaxis, ideally before 3 years of age and prior to the onset of joint disease, with either standard or extended half-life FIX concentrates or other hemostatic agents.[2] Despite the preference for recombinant FIX products, plasma-derived FIX concentrates such as IMMUNINE® remain a viable and effective therapeutic option for the management of patients with hemophilia B, especially in countries where treatment options are more limited. In this PASS study, purified FIX concentrate was not associated with any treatment-related AEs and was shown to be effective in treating and preventing bleeding episodes in pediatric patients ≤6 years old with hemophilia B. The findings from this study, therefore, provide further support to the continued use of plasma-derived FIX concentrates such as IMMUNINE® in the management of patients with hemophilia B.

Abbreviations

AE, adverse event; BU, Bethesda units; ED, exposure day; FIX, factor IX; ITT, intent to treat; PASS, post-authorization safety surveillance; PP, per protocol; SAE, serious adverse event; SD, standard deviation; WFH, World Federation of Hemophilia.

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 3 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.

Ethics Approval and Informed Consent

The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice E6, Title 21 of the US Code of Federal Regulations, the European Clinical Trial Directive, and applicable national and local regulatory requirements, including European regulations pertaining to PASS studies. This study was also conducted in accordance with the Declaration of Helsinki. Approval from Institutional Review Boards/Independent Ethics Committees, listed in Supporting Information, was obtained for the study protocol and informed consent form. All patients or their legally authorized representative signed an informed consent form prior to entering the study.

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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.

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Disclosure

Zoran Igrutinović reports being an investigator for this post-authorization safety surveillance study, which was funded by Baxalta Innovations GmbH, Vienna, Austria, a Takeda company. Hélène Louise Hooimeijer has no interests that might be perceived as posing a conflict or bias for this study. Karim Kentouche reports being an investigator for this post-authorization safety surveillance study, which was funded by Baxalta Innovations GmbH, Vienna, Austria, a Takeda company. Jaco Botha and Marta Kokot-Kierepa are employees of Takeda Pharmaceuticals International AG, and Takeda stockholders. Peter L. Turecek is an employee of Baxalta Innovations GmbH, a Takeda company, and a Takeda stockholder. Hanna T. Gazda is an employee of Takeda Development Center Americas, Inc., a Takeda company, and a Takeda shareholder. The authors report no other conflicts of interest in this work.

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Case Study: Rosai-Dorfman Disease and Its Multifaceted Aspects

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CASE SERIES

Case Study: Rosai-Dorfman Disease and Its Multifaceted Aspects

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Abstract: Rosai-Dorfman Disease (RDD) is a rare non-Langerhans histiocytosis, usually self-limited and presenting with massive, painless, bilateral cervical lymphadenopathy, with or without constitutional symptoms. Extranodal disease is frequently present, and may happen in the absence of lymph node involvement, symptomatology and differential diagnosis will depend on the site affected and fatal cases may occur. The authors present two cases of Rosai-Dorfman disease (RDD), diagnosed through immunohistochemistry, with different progressions, one with complete remission and one culminating in death, highlighting the variety of presentations and the diagnostic difficulty. RDD is a rare condition with clinical presentations similar to several diseases, and should be considered in the differential diagnosis of lymphadenopathy with extranodal lesions.

Keywords: Rosai Dorfman disease, immunohistochemistry, diagnosis, differential diagnosis (MeSH-NCBI)

Introduction

Rosai-Dorfman disease (RDD) was first described by Destombes in 1965, and later by Rosai and Dorfman in 1969, as a sinus histiocytosis with massive lymphadenopathy.[1–3] It is a rare non-Langerhans histiocytosis, with a prevalence of 1:200,000 and an annual incidence of 100 cases per year in the United States.[1] It predominantly affects males (1.4:1), children and young adults, with a mean age of 20.6 years. RDD is classified as sporadic (classic nodal or extranodal), familial or cutaneous, and may be associated with onco-hematological neoplasms and autoimmune diseases (Figure 1).[4] The disease is usually self-limited and presents with massive, painless, bilateral cervical lymphadenopathy, with or without constitutional symptoms. The prognosis is associated with the number of lymph node groups involved and the affected site, the clinical course is chronic and may alternate between episodes of exacerbation and remission.[5] Its etiology and pathogenesis are unclear, and probably are not uniform across the disease spectrum, initially it was classified as a polyclonal, non-neoplastic and reactive histiocytosis, occurring from an aberrant immune response associated with infections, but studies have failed to establish a causal link, and current research may lead to future changes in disease classification.[5]

New studies show evidence of a clonal nature in a subset of cases, generally caused by mutations in the MAPK pathway, most frequently in the MAP2K1 and KRAS genes.[6] However, Durham et al performed whole exome sequencing in 17 cases of RDD and found alterations not only in the kinases of the MAPK pathway, but also in genes involved in several intracellular pathways, such as intracellular trafficking, transcriptional regulation, cell cycle regulation, DNA mismatch repair and the ubiquitin proteasome pathway.[7] BRAF V600E mutations, often described in LCH, are rare but not absent in RDD, in a total of 94 cases reviewed in the literature, only 3 had alterations in the gene.[1,8]

The diagnosis of RDD is difficult and often delayed due to its rarity, varied presentation, and nonspecific symptoms.[5,9,10] Laboratory evaluation may show normocytic and normochromic anemia, leukocytosis, thrombocytopenia, eosinophilia,

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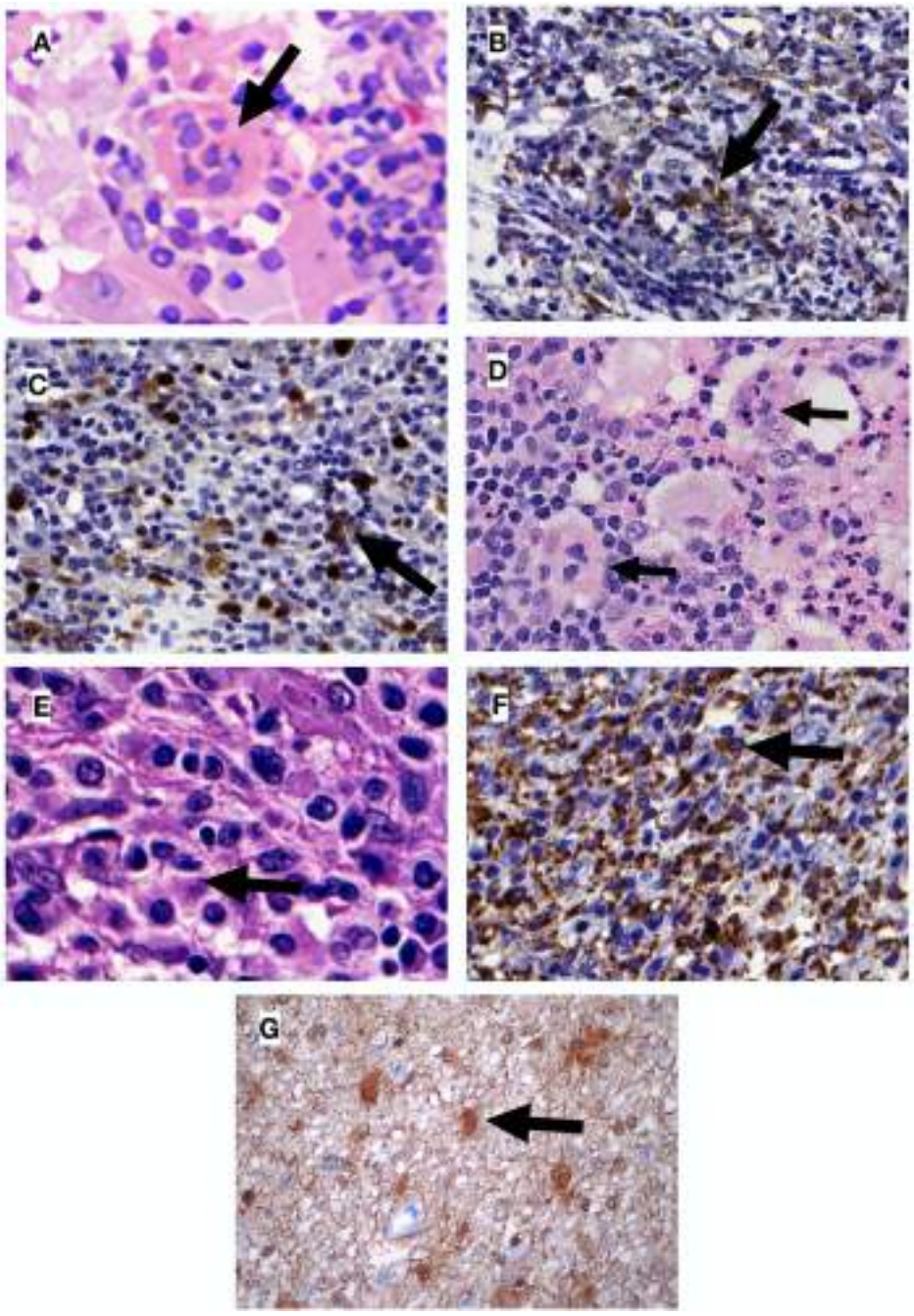

Figure 1 Histopathological findings for patients 1 and 2. (**A**) Patient 1. Non-neoplastic proliferation showing lymphocyte emperipolesis on 800x increase; (**B**) Patient 1. Positive immunohistochemistry for CD68 (indicating macrophages) on histiocytic infiltrate; (**C**) Patient 1. Immunohistochemistry with occasional positivity for S100 on histiocytic infiltrate; (**D**) Patient 2. Non-neoplastic proliferation showing lymphocyte emperipolesis on 400x increase; (**E**) Patient 2. Non-neoplastic proliferation showing lymphocyte emperipolesis on 800x increase; (**F**) Patient 2. Positive immunohistochemistry for CD68 (indicating macrophages) on histiocytic infiltrate; (**G**) Patient 2. Positive immunohistochemistry for S100; Black arrows indicate emperipolesis on (**A, D** and **E**), and indicate immunohistochemistry positivity on (**B, C, F** and **G**).

hypergammaglobulinemia, and elevated ESR. Intermittent fever, night sweats, weight loss, and asthenia may be present. In the pathology, there is a marked sinusoidal expansion, which may lead to the disappearance of the nodal architecture, with a diffuse infiltrate of histiocytes with pale and ill-defined cytoplasm, and a presence of plasma cells, which may be positive for IgG4, a factor with not yet defined importance.[1] The characteristic finding is emperipolesis, defined as an intact hematolymphoid cell vacuolated or floating freely in the cytoplasm of macrophages. RDD immunohistochemistry

histiocytes are positive for S100, CD68, CD163 and negative for CD1a and CD207, excluding Langerhans-cell histiocytosis (LCH), the main differential diagnosis of RDD.[1] Other diseases considered in the differential diagnosis of RDD are Sinus histiocytosis, a reactive process characterizes by a positivity for S-100, cyclin D1 and negative for OCT2; Erdheim-Chester disease (EDD), Juvenile xanthogranuloma, which both show abundant foamy cytoplasm with surrounding fibrosis, EDD's histiocytes are positiver for CD68, CD163, factor XIIIa and negative for OCT2; and low grade B cell lymphoma, which is negative for OCT2. The following case reports have been approved by the Ethics Committee of the care providing institution, and all patients provided written informed consent for their personal or clinical details along with any identifying images to be published in this study.

Case Reports

Case 1

A 57-year-old white woman presented, in April 2007, with a nodular cutaneous lesion in the left breast, measuring 1.2 cm in the longest axis, without phlogistic signs or constitutional symptoms, during a routine primary care visit. Mammography and ultrasound of the breast were performed, with normal results. After nodulectomy with 2.5cm margins, the histopathological report showed histiocytic proliferation and emperipolesis. Immunohistochemistry was positive for anti-protein S-100 and anti-CD68 antibodies and negative for anti-CD1a, confirming the diagnosis of cutaneous RDD (Figure 1). Immunohistochemical panels were performed for breast cancer humoral receptors (ER, RP and HER 2) that were non-reactive. The patient was referred to the hematology service.

A chest and abdomen computed tomography for investigation of nodal disease was carried out, which did not identify other sites of disease. Considering the laboratory (Table 1), radiological and clinical data, we opted for an expectant management.

Table 1 Laboratory and Microbiology Results from Patients 1 and 2

Laboratory Values and Microbiology	Patient 1	Patient 2 Admission	Patient 2 Readmission
Aspartate aminotransferase, U/L	36	44	158
Alanine aminotransferase, U/L	28	40	86
Gamma-glutamyltransferase, U/L	18	588	130
Alkaline phosphatase, U/L	94	194	462
Total bilirubin, mg/dL	1.0	1.8	7.4
Direct bilirubin, mg/dL	0.2	0.8	5.1
Indirect bilirubin, mg/dL	0.5	0.6	2.3
Lactic dehydrogenase, U/L	316	422	892
Creatine phosphokinase, U/L	–	404	–
Erythrocyte sedimentation rate, mm/h	11	56	80
Prothrombin time, seconds	100	–	27.5
Beta-2 microglobulin, mg/L	2.1	3.3	–
Total proteins, g/dL	6.8	7	7.2
Albumin, g/dL	4	3	2.6
Globulin, g/dL	2.8	4	4.6
Amylase, U/L	–	40	178
Lipase, U/L	–	–	91
Ferritin, ng/mL	47.4	480	–
Triglycerides, mg/dL	–	91	–
Total cholesterol, mg/dL	–	158	–
Serum copper, µg/dL	100	105	–
Creatinine, mg/dL	0.8	1.0	2.4
Urea, mg/dL	24	47	104
Potassium, mEq/L	4	4	7.5

(*Continued*)

Table 1 (Continued).

Laboratory Values and Microbiology	Patient 1	Patient 2 Admission	Patient 2 Readmission
pH	–	–	6.9
pCO2, mmHg	–	–	7
Bicarbonate, mEq/L	–	–	35
BE, mEq/L	–	–	−26
SaO2, %	–	–	82
Blood cultures	–	–	Neg
Human immunodeficiency virus antigen/antibody (HIV)	Neg	Neg	–
Venereal Disease Research Laboratory (VDRL)	Neg	Neg	–
Hepatitis B surface antigen	Neg	Neg	–
Hepatitis C antibody	Neg	Neg	–
Epstein-Barr antibody	–	Neg	–
Human T-lymphotropic virus - 1/2 antibody (HTLV-1/2)	Neg	Neg	–

Abbreviations: pH, potential of Hydrogen; pCO2, partial pressure of carbon dioxide; BE, base excess; SaO2, arterial oxygen saturation; neg, negative.

In April 2008, a new nodule measuring 1.5 cm X 1.0 cm was observed on the excisional biopsy scar, the patient underwent a new biopsy with 2.5 cm margins, another body CT, and a bone marrow study, again showing cutaneous RDD, with S-100 positivity and negative for CD1a, with no extranodal disease.

The patient remains under outpatient onco-hematological control and has been in clinical remission for 14 years, with the last consultation in October 2022.

Case 2

A 26-year-old man of African descent was referred to the Oncological Surgery Service in January 2010, with painful right inguinal adenomegaly (3.5 X 2.5×1.5 cm), with progressive growth for 20 days, without phlogistic signs. The patient reported continuous 39°C fever, asthenia and hyporexia.

Upon admission, the patient was anemic, and had increased erythrocyte sedimentation rate (ESR), creatine phosphokinase (CPK), gamma glutamyl transferase (GGT) and alkaline phosphatase (AP) (Table 1). There was a suspicion of lymphoproliferative disease, and a biopsy was performed with subsequent discharge. However, the patient's symptomatology worsened and he was readmitted. Abdominal ultrasound showed splenomegaly (16.5 cm in the longest axis) and multiple enlarged hypoechoic lymph nodes (2.5 cm X 3 cm) in the right iliac fossa and retroperitoneum. Posteroanterior chest X-ray revealed heterogeneous opacity with a nodule in the hilar and right paracardiac regions, in addition to mediastinal right inter-cleido-hilar widening. Considering the severity of the case, prednisone, ceftazidime and gentamicin were empirically started before a definitive diagnosis. The workup for infectious diseases was negative. The patient's general condition worsened, with generalized adenomegaly, sepsis, hepatic and renal failure, dying 15 days after the biopsy.

Histopathology of the lymph node identified a proliferative process of undetermined nature, with rounded cells and wispy cytoplasm, requiring immunohistochemistry for diagnostic definition, which confirmed the replacement of normal lymph node architecture by histiocyte proliferation, with lymphoplasmacytic infiltrate and emperipolesis, with positivity for S-100, CD68, and sparse CD45, and negative for CD1a, CD207, CD30 (K1-1), clone Ber-H2, CD21 (clone 1F8), CD20 (clone L-26), CD3 (policlonal), CD15 (clone C3D-1), CD117 (clone T595), CD34 (clone BIRMA-K3), CD23 (clone MHM6) (Figure 1). A presumptive diagnosis of RDD was established post mortem.

Discussion

The cases presented demonstrate the variable manifestations and evolution of RDD. In the first case, there was cutaneous involvement in the left breast, without lymph node disease or other site of injury, with recrudescence after surgical

Table 2 Differential Diagnosis for Rosai-Dorfman Disease

Differential Diagnosis in RDD	
C group histiocytoses	*Malignancies*
Eruptive xanthoma	Malignant lymphoma
Juvenile xanthogranulomatoma	Metastatic melanoma
Solitary reticulohistiocytoma	Sarcomas and Stewart-Treves Syndrome
L group histiocytoses	Anaplastic large cell lymphoma
Langerhans cell histiocytosis	Other metastatic neoplasias
Erdheim-Chester disease	Autoimmune disorders
H group histiocytoses	Castleman disease
Hemophagocytic syndrome	IgG4 disease
Infectious lesions	*Gaucher disease*
Acquired Immunodefficiency Syndrome	Whipple *disease*
Granulomatosis with polyangiitis	*Benign soft tissue neoplasias*

excision, and complete remission after a second excision. This behavior is typical of the cutaneous involvement of RDD, an article that followed 202 patients showed that after surgical excision, about 40% of patients do not achieve complete remission. However, the disease remained localized and did not present malignant evolution.[11]

Extranodal disease is present in up to 43% of all RDD cases, and may occur in the absence of lymph node involvement. The sites most commonly affected are skin (10%), nasal cavity and paranasal sinuses (11%), bone (5–10%), orbital tissue (11%) and central nervous system (5%).[1,5] Symptomatology and differential diagnosis will depend on the site affected (Table 2). Cutaneous RDD is considered a distinct clinical entity, being more common in women (2:1) around 43.5 years, with a higher incidence in Caucasians and Asians, it is generally not associated with systemic or extracutaneous disease, and tends to remain localized, even after long periods of evolution.[11]

In the second case, there was an aggressive presentation, with multifocal lymphadenopathy, intense constitutional symptoms and laboratory markers indicating severe liver and pancreatic damage, with initial suspicion of non-Hodgkin's lymphoma, and rapid progression to death. The aggressiveness of a normally benign disease, the absence of risk factors or associated diseases show a case of exception. Extranodal disease, particularly the intracranial phenotype, is associated with a poor prognosis, deaths in RDD occur due to direct complications of the disease, infections and amyloidosis. There was not enough time to carry out an adequate investigation of an extranodal condition, and the real cause of splenomegaly and multi-organ failure is not definitively known. Several conditions were considered in the differential diagnosis, such as Langerhans cell histiocytosis, Erdheim-Chester disease and Hemophagocytic Lymphohistiocytosis (HLH), however, the immunohistochemistry result suggested RDD. The serology for Epstein-Barr, an infection commonly associated with HLH, was negative. Thus, based on the patient's initial clinical manifestations and immunohistochemistry results, the possibility of liver and kidney involvement is considered in an attempt to justify the unfavorable evolution.

Sporadic RDD spontaneously remisses in up to 50% of all cases, mortality is around 10%, occurring due to direct complications of the disease, infections and amyloidosis. Extranodal disease presenting as intracranial or mediastinal RDD seems to have a poorer prognosis, being responsible for 50% of the deaths in a literature review, nosocomial infections, kidney RDD and generalized amyloidosis were also mentioned as the cause of death in case reports.[1,12–14]

Expectant management is recommended for oligosymptomatic patients and those with unifocal cutaneous RDD. Surgical excision is usually restricted for patients with unifocal extranodal disease. Patients with unresectable multifocal extra nodal RDD and disease affecting airways, intracranial or intraspinal space require systemic therapy, which remains without a standardized regimen, and can include corticosteroids, sirolimus, radiotherapy, chemotherapy (cladribine, methotrexate, 6-mercaptopurine, vinca alkaloids), and immunomodulators (thalidomide, lenalidomide, rituximab, imatinib mesylate).[1,5]

Conclusion

RDD is rare and presents in a multisystemic form, requiring its inclusion in the differential diagnosis of suspected lymphoproliferative and histiocytic diseases, as well as in skin lesions with difficult characterization and fever of unknown origin.

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Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Prevalence and Associated Factors of Anemia among Newborns at Jimma Medical Center, South-west Ethiopia

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Background: Newborn anemia is among the most common hematological problems and it can cause asymptomatic or severe to acute life-threatening events. It leads to impairment in brain maturation and development, tissue hypoxia, and stunted growth and then arrested growth if left untreated. The prevalence of anemia among newborns ranges from 23.4–66% in sub-Saharan Africa. But, there is limited information in Ethiopia regarding the prevalence of newborn anemia and its risk factors. Therefore, this study aimed to determine the prevalence of newborn anemia and its associated factors at Jimma Medical Center (JMC), South-west Ethiopia.

Methods: A hospital-based cross-sectional study design was implemented from January 14 to February 28, 2021, involving 288 full-term newborns by employing consecutive convenient sampling technique for study participant selection. Socio-demographic data and other associated factors were collected through interviews and a review of medical records by a structured questionnaire. Three mL umbilical cord blood samples from each newborn were collected and analyzed for a complete blood count by an automated hematological analyzer. Data were entered into Epi Data version 3.1 and exported to Statistical Package for Social Science version 20 for analysis. Binary logistic regression were used to identify the predictors of newborn anemia.

Results: The overall prevalence of anemia among newborns was 26.4%; of them, 65.8%, 25%, and 9.2% were mild, moderate, and severe anemia types, respectively. Maternal vegetable consumption habit (AOR = 0.26, 95% CI: 0.11, 0.62) and maternal anemia (AOR = 0.34, 95% CI: 0.17, 0.69) were significantly associated with anemia in newborns.

Conclusion: In general, newborn anemia in this study was a moderate public health problem. Based on this study, early screening of anemia among newborns may reduce further complications. Prevention of maternal anemia during pregnancy by improving their nutritional status especially vegetable consumption had a positive impact on reducing anemia among newborns.

Keywords: newborn, anemia, Jimma, Ethiopia

Introduction

Anemia is defined by the World Health Organization as a reduction in the amount of red blood cells in circulation, which causes the hemoglobin (Hgb) concentration to drop below the necessary level. This impairs the body's ability to transport oxygen and is insufficient to meet its physiological requirements. Specific physiologic needs vary with a person's age, gender, altitude, smoking behavior, and different stages of pregnancy.[1]

Anemia in newborns is defined as a condition in which hemoglobin levels in the body are lower than normal. However, there is no established Hgb limit for cord blood to define anemia in newborns; this study used an Hgb value of less than 14 g/dL, adopted from a study conducted in Ethiopia,[2] then anemia in newborns or neonate is divided into three categories based on hemoglobin value as follows: 10 to 13.9 g/dl for mild anemia, 7 to 9. 9 g/dl for moderate anemia and < 7 g/dl is severe anemia. Additionally, maternal anemia is defined as Hgb <11g/dL.[3,4]

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In developing countries, the prevalence of newborn anemia and severe anemia were between 50% and 80%, and 10% to 20%, respectively.[5] Because it causes infant mortality and morbidity in the first few months of a baby's life, newborn anemia is a severe public health concern.[5–7] Its prevalence in sub-Saharan Africa is high, ranging from 23.4% to 66%.[8,9]

The study carried out in Ethiopia indicated that the prevalence of neonatal anemia of 9%, 25%, and 29.1% were reported in Addis Ababa, Gondar, and Nekemte, respectively. Furthermore, those studies found that anemia in the mother, mothers' age, preterm birth, her habit of consuming vegetables, cesarean delivery, occupation, parasitic infection, vaginal bleeding during pregnancy and her failure to take iron/folate supplements during her pregnancy were all associated with newborn anemia.[10–12]

In a newborn, anemia is a common and complex problem owing to the unique blood picture because of variations in hematological profiles.[13] It may result in an acute, severe, or asymptomatic life-threatening event.[14] Anemia negatively impacts a newborn's short- and long-term mental, physical, and social development. It also affects brain development, delays brain maturation, impairs later-life school performance, stunts growth, causes tissue and organ hypoxia, and worsens cognitive, motor, and social-emotional development. These effects lower earning potential and have a negative impact on the nation's economy.[6,15] Additionally, it is worse in developing nations with low iron intake, ineffective interventional programs, and inadequate infrastructure for early detection and treatment of anemia in health care facilities.[16,17]

Due to the increased metabolic needs of pregnancy following expanding placenta, a growing fetus, and maternal tissues, pregnant women are especially more susceptible to iron deficiency. It thus raises the possibility of maternal anemia. Since the fetus depends on the mother's iron levels; women at greater risk of iron deficiency stand a higher chance of having anemic newborns. Because of these, anemia in newborns presents severe public health problems and results in newborn morbidity and mortality in underdeveloped nations.[18–21]

Although anemia's national and regional prevalence in various population age groups is known in Ethiopia,[4] there is a dearth of information regarding the severity of anemia and its contributing factors in neonates. To prevent, treat, and reduce anemia, it is crucial to research the particular risk factors and prevalence of anemia in a given setting and population group.[3] Thus, the purpose of this study was to assess the prevalence of newborn anemia and its associated risk factors at Jimma Medical Center (JMC), south-west Ethiopia.

Materials and Methods

Study Design, Period and Area

A hospital-based cross-sectional study was conducted from January 14 to February 28, 2021 at JMC's delivery ward. The medical center is found at Jimma town, located from 350 km in the south-west of Addis Ababa, the capital city of Ethiopia. The location of the area is 1,780 meters above sea level, with approximate latitude coordinates of 7° 40'N and longitude of 36° 50'E. JMC provides services for approximately 4,500 deliveries in a year and 15 deliveries are expected daily from the catchment population of about 20 million people.

Study Participants

All mothers with their respective singleton live birth newborns delivered at full-term gestation (37–41 weeks) at JMC during the study period were included in the study. On the other hand, newborns with inaccessible cord blood samples, pregnant mothers who had taken blood and blood products for the last 3 months, who were receiving therapy for anemia, and had taken anti-parasite drugs in the previous two weeks were not included in this study.

Sample Size Determination and Sampling Technique

The minimum sample size was determined using single population proportion formula $n = (Z\alpha/2)2(p(1-p))/d^2)$ by considering the following assumptions, taking 25% prevalence of newborn anemia from the previous study done at University of Gondars' specialized hospital, Ethiopia.[11] Therefore, the final minimum sample size was 288. Mothers with their respective newborns were recruited by consecutive convenient sampling technique.

Data Collection Methods

A pre-tested structured questionnaire and reviews of medical records using a checklist were used to collect the data. The questionnaire was prepared after reviewing different related literature inside[10,11] and outside the country.[16,22,23] The questionnaire, first prepared in English language was translated into local language, then it was translated back to English language to ensure its consistency. Maternal socio-demographic characteristics, nutritional characteristics, birth interval, history of intestinal parasite infection (Helminths and protozoan parasite infection diagnosis during pregnancy) and malaria diagnosis during pregnancy, history of miscarriage, history of excess menstrual bleeding, and newborn gender were collected by face-to-face interviews and also by reviewing medical records.

Regarding nutritional characteristics or diet information of the mother, the women were asked about the frequency of consumption of each food (meat or animal product, vegetable and fruit consumption) per day, per week, or per month. In this study, pregnant women were defined as "consumers" of a food item (meat or animal product, vegetable and fruit consumption) when they had consumed those items at least once a week or per week.[24,25]

In this study vaginal bleeding was referred to as bleeding that was not related to menstruation. Menstrual bleeding was assessed based on asking the mother about the average length of menstruation or assessed by using an average number of sanitary pads changed per day. Menstrual bleeding was considered as excessive when the average length of menstruation was longer than 7 days or an average number of sanitary pads changed per day was greater than 4.

Maternal clinical data, CBC result, and blood group type were collected by reviewing medical records. For mothers who had no CBC results that was performed when the mothers come to delivery and have no blood group records, CBC test and blood grouping was performed by collecting 3ml venous blood. The interview, record review and blood collection were conducted by two trained midwifery professionals.

ABO blood incompatibility was defined in this study when group A or B babies born to group O mothers and also RH blood incompatibility occurs when Rh-positive babies were born to Rh-negative mothers.

The nutritional status of the pregnant mothers was determined by Mid-Upper Arm Circumference (MUAC) between the shoulder (olecranon) and the tip of the elbow (acromion process) of the non-dominant hand using a non-stretchable and non-elastic tape then, the result was interpreted as undernourished and well-nourished if her MUAC was less than 23 cm and greater than or equal to 23 cm respectively.[26,27] Gestational age was determined by the last menstrual period but the last menstrual period of the mother was not known, gestational age was estimated by the New Ballard Score for inclusion and exclusion of study participants. The data collectors measured the newborn's weight using a digital scale and the measurement was performed twice and an average value was taken. A newborn's weight greater than or equal to 2.5 kg was considered as normal birth weight and less than 2.5 kg was considered as low birth weight.[4]

About three[3] ml of umbilical cord blood sample was collected after the baby was born and the placental side of the umbilical cord was clamped and cut (in <2 minute after birth), and then needle was inserted into pre-identified large vein of the clamped umbilical cord by excluding the placenta. The collected sample was immediately poured into a tri-potassium ethylene diamine tetra-acetic acid (K3-EDTA) test tube and gently mixed to prevent blood clotting. The specimen was labeled and analyzed for CBC within 3–6 hours using Beckman coulter (UniCel® DxH 800, Florida, USA) hematological analyzer machine based on direct current principle for cells and spectrophotometric principle for Hgb. ABO blood group and Rh type of the newborn were determined from blood sample through forward (direct method) using test tube method. Two experienced laboratory technologist performs the CBC and blood grouping by strictly following to standard operating procedures.

Umbilical cord blood hemoglobin value is a dominant hematologic parameter for the diagnosis of newborn anemia.[28,29] Hence this parameter was used in this study to define anemia in newborns. In this study, newborn anemia was defined as Hgb value <14g/dL and 10 to 13.9 g/dl as mild anemia, 7 to 9. 9 g/dl as moderate anemia and < 7 g/dl as severe anemia. Also maternal anemia was defined as Hgb <11g/ dL.

Data Quality Assurance

To assure the quality or validity of the data, the questionnaire was pre-tested among 5% of the sample size at Shenen Gibe Hospital, Jimma. The reliability of the tool was determined using Cronbach's Alpha. Cronbach's alpha among items in these study questionnaires was 0.892, which exceeded the prescribed threshold of 0.70. Training was given to data collectors, daily cross-checking of collected data with maternal records and daily supervision was made during the data

collection period to assure the quality of socio-demographic data, clinical data and laboratory data. Standard operating procedures were also strictly followed during specimen collection and CBC analysis.

A daily background run for the hematological analyzer was carried out to minimize errors. Reagent expiration date was checked before the analysis of the patient sample and appropriate internal quality controls were run before the assay of samples. The test findings were kept confidential. The results of every laboratory test were documented and reported, and the specimens were handled carefully.

Data Processing and Analysis

The data from both questioner and laboratory were cleaned, edited, checked for completeness manually, and entered into Epi Data 3.1. Then it was transferred into a SPSS version 20 for analysis. Prior to any analysis, the Shapiro–Wilk and Kolmogorov–Smirnov tests were used to confirm that the data were normally distributed. For each independent variables, descriptive analysis were performed and summarized by number, percentage and frequency. The result was also presented with tables and charts. Bivariable logistic regression was performed to identify candidate variables and then variables having p-value less than or equal to 0.25 were candidate for multivariate logistic regression. Multivariate logistic regression was analyzed with backward stepwise likelihood ratio to assess association outcome with independent variable and to control potential confounding variables and then a p-value <0.05 were considered as having a significant relationship with the dependent variables. The Hosmer and Lemeshow test was used to assess the model fitness of the final logistic regression model at a p-value of greater than 0.05.

Results

Socio-Demographic Data of Study Participants

Two hundred eighty-eight (288) newborns, which comprised142 males and 146 females, and their respective mothers were include in the study. The median (IQR) age of the mothers was 25 (23-33) years with the minimum and maximum ages of 18 and 38 years, respectively. About 72.5% of newborns were delivered from maternal age below 30 years. About 72.9% of newborns were delivered through spontaneous vaginal delivery and the majority (88.5%) newborns had normal birth weight. Of the total newborns, 75% and 39.9% were born from mothers living in an urban area and who were housewives by their occupation, respectively. In addition, 80.9% of newborns were born from mothers having >1000ETB family monthly income (Table 1).

Clinical Data of Study Participants

All study participant mothers were HIV negative. Of the total study participant, 62.5% were born from multiparous mothers and 64.4% from mothers were greater than 2 years birth interval. In this study, about 9.7% of babies were born to mothers who had no antenatal care follow-up. The majority of mothers (83.3%) had taken iron and folic acid supplementation, mainly (53.3%) at the second trimester of pregnancy. Of the total mothers, 10.1%, 16.7%, and 6.9% had a history of vaginal bleeding, intestinal parasitic infection, and a history of malaria during their pregnancy, respectively (Table 2).

Nutrition Data of Study Participants

More than two-thirds (68.4%) of birthing mothers had meal frequency greater than three times per day. However, only 11.5% of mothers were undernourished. More than 84% of the newborns were delivered by mothers who consume meat or animal products, vegetables, and fruit every week. The maternal Hgb value ranges from 6.8–16.8 g/dl with a median (IQR) value of 13 g/dl (12–14.2g/dl). In this study, 58 (20.1%) mothers were anemic and from this 4 (6.9%), 15 (25.9%), and 39 (67.2%) were severe, moderate, and mild anemia type, respectively (Table 3).

Prevalence of Newborn Anemia

The cord Hgb value ranges from 4.2–21.5 g/dl with a median (IQR) value of 15.8 g/dl (13.8–16. 9 g/dl). The overall prevalence of anemia among full-term newborns at JMC was 26.4% (76/288) and from anemic newborn, 65.8, 25 and 9.2% were mild, moderate, and severe anemia respectively (Figure 1).

Table 1 Socio-Demographic Characteristics of Study Participants at JMC, Southwest Ethiopia from January 14 to February 28, 2021 (n = 288)

Maternal and Newborn characteristic		Frequency	Percentage (%)
Newborn gender	Male	142	49.3
	Female	146	50.7
Newborn birth weight	Low birth weight	33	11.5
	Normal birth weight	255	88.5
Maternal age	≤24 years	94	32.6
	25–29 years	115	39.9
	≥ 30 years	79	27.4
Residence	Rural	72	25
	Urban	216	75
Maternal occupation	Housewife	115	39.9
	Small scale business	91	31.6
	Employed	82	28.5
Maternal education level	No formal education	64	22.2
	Primary education	69	24
	Secondary education	88	30.6
	Above secondary education	67	23.3
Family monthly income	<500 ETB	20	6.9
	501–1000 ETB	35	12.2
	>1000 ETB	233	80.9

Based on red blood cell indices especially MCV value (normal range: 91.6–113.22),[2] microcytic, normocytic and macrocytic anemia among newborns were 7.9%, 31.6% and 60.5% respectively. Additionally, about 13 (17.1%) of newborns abies were anemic having ABO incompatibility with their respective mothers but 8 (2.8%) newborns having Rhesus incompatibility with their respective mothers were non anemic due to properly managed during ANC follow up.

Associated Factors of Newborn Anemia

Newborn anemia was high in those babies born from mothers who do not consume vegetables (51.5%), who were anemic (39.7%), who do not take iron or folic acid supplementation (39.6%), who do not consume meat or animal product (37.8%) and who do not follow ANC (35.7%). Based on bivariable logistic regression analysis, maternal consumption habit of vegetable, consumption habit of fruit, iron or folic acid supplementation and maternal anemia were showed significant association with newborn anemia. However, in multivariate binary logistic regression analysis, only mothers' vegetable consumption habits (AOR = 0.26, 95%CI: 0.11, 0.62) and maternal anemia (AOR = 0.34, 95%CI: 0.17, 0.69) were significantly associated with newborn anemia (Table 4).

Discussion

The overall prevalence of newborn anemia in this study was 26.4% (76/288) and according to WHO public health limits, this result showed that newborn anemia was a moderate public health problem at JMC.[3] Several study results reported

Table 2 Clinical Characteristics of Study Participants at JMC, South-west Ethiopia from January 14 to February 28, 2021 (n = 288)

Variables		Frequency	Percent (%)
Parity	Primiparous	108	37.5
	Multiparous	180	62.5
Birth interval (n = 180)	≤ 2	64	35.6
	>2	116	64.4
ANC follow up	Yes	260	90.3
	No	28	9.7
Iron and folic acid supplementation	Yes	240	83.3
	No	48	16.7
Trimester iron or folic acid supplementation started (n = 240)	First trimester	84	35
	Second Trimester	128	53.3
	Third trimester	28	11.7
History of miscarriage	Yes	19	6.6
	No	269	93.4
History of excess menstrual bleeding	Yes	33	11.5
	No	255	88.5
History of vaginal bleeding during pregnancy	Yes	29	10.1
	No	259	89.9
History of intestinal parasite infection during pregnancy	Yes	48	16.7
	No	240	83.3
History of malaria infection during pregnancy	Yes	20	6.9
	No	268	93.1
Rhesus incompatibility managed	Yes	8	2.8
	No	280	97.2
ABO incompatibility	Yes	47	16.3
	No	241	83.7
Current mode of delivery	Spontaneous vaginal	210	72.9
	Cesarean section	78	27.1

from different countries such as the Netherlands (21%),[30] Brazil (32.6%),[22] New York, US (21%),[31] Nigeria (34.5%)[32] and in Gondar, Ethiopia (25%)[11] was consistent with this study result.

However, compared to a study conducted in Addis Ababa, Ethiopia, which found a 9% prevalence, the anemia prevalence among newborns in the current study was higher.[10] Sample size variations and study population differences could be the cause of this discrepancy. In the present study, 288 study participants were included, whereas only 89 study participants were included to a study from Addis Ababa. Regarding study population difference, the study conducted from Addis Ababa excludes newborns from mothers who had a history of vaginal bleeding during pregnancy but in this

Table 3 Nutrition Characteristics of Study Participants at JMC, South-west Ethiopia from January 14 to February 28, 2021 (n = 288)

Nutritional Characteristics		Frequency	Percent (%)
Meal frequency within a day	>3 times per day	197	68.4
	3 times per day	78	27.1
	≤ 2 times per day	13	4.5
Meat or animal product consumption per week	Yes	243	84.4
	No	45	15.6
Vegetable consumption per week	Yes	255	88.5
	No	33	11.5
Fruit consumption per week	Yes	260	90.3
	No	28	9.7
Nutritional status (MUAC)	Malnourished	33	11.5
	Normal	255	88.5
Maternal anemia	Yes	58	20.1
	No	230	79.9

study, 29 newborns from mothers who had a history of vaginal bleeding during pregnancy were included and this may increase the prevalence of anemia in the current study. Besides which, anemia prevalence in our study was higher than a report from Nepal (5.7%),[28] the US(14%),[33] and Iran (5.8% and 11.7%).[34,35] The observed variation in the prevalence of anemia among the studies could potentially be attributed to factors such as low socioeconomic status, inadequate prenatal care coverage and quality, and clinical characteristics of the current study participants relative to those in the aforementioned countries.

On the other hand, the prevalence of anemia in the current study was lower than in the previously published study from Iran (53%),[36] Benin (61.1%),[37] Nigeria (65.6%),[38] and Ghana (57.3%).[23] The possible reason for the difference of this study from Iran could be ascribed to differences in research subjects way of delivery. The majority of our study participants (210/ 72.9%) were born vaginally and because of this 45 (59.2%) newborns were anemic, as well as 78 (27.1%) who were born through cesarean section and of these, 31 (40.8%) newborns were anemic but all study

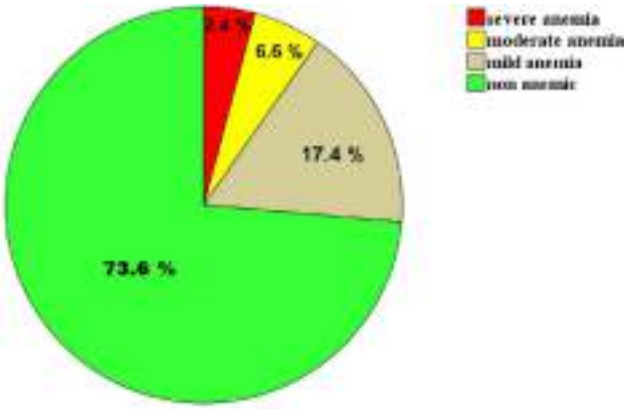

Figure 1 Prevalence of mild, moderate, and severe anemia in the total newborn babies at JMC, South-west Ethiopia from January 14 to February 28, 2021 (n = 288).

Table 4 Bivariable and Multivariable Binary Logistic Regression Analysis of Factors Associated with Anemia Among Newborn Babies at JMC, South-west Ethiopia from January 14 to February 28, 2021 (n = 288)

Characteristics of Study Participants		Anemic Newborns	Non-anemic Newborns	COR (95%CI)	AOR (95%CI)	P-value
Maternal age	≤24 year	28(29.8%)	66(70.2%)	1	1	0.242
	25–29 year	31(27%)	84(73%)	0.87(0.48–1.59)	1.10(0.52–2.30)	
	≥30 year	17(21.5%)	62(78.5%)	0.65(0.32–1.30)	0.54(0.22–1.30)	
Residence	Rural	26(36.1%)	46(63.9%)	1.88(1.06–3.34)	0.87(0.28–2.74)	0.809
	Urban	50(23.1%)	166(76.9%)	1	1	
Maternal education level	No formal education	24(37.5%)	40(62.5%)	2.75(1.23–6.14)	3.43(0.87–13.5)	0.295
	Primary school	18(26.1%)	51(73.9%)	1.62(0.71–3.69)	2.97(0.83–10.6)	
	Secondary school	22(25%)	66(75%)	1.53(0.69–3.36)	1.86(0.54–6.37)	
	Above secondary school	12(17.9%)	55(82.1%)	1	1	
Maternal occupation	Housewife	34(29.6%)	81(70.4%)	1.73(0.88–3.41)	1.28(0.56–2.94)	0.171
	Small scale business	26(28.6%)	65(71.4%)	1.65(0.81–3.36)	2.14(0.94–4.88)	
	Employed	16(19.5%)	66(80.5%)	1	1	
Family monthly income	<500 ETB	9(45%)	11(55%)	2.65(1.04–6.72)	1.77(0.50–6.28)	0.533
	501–1000 ETB	12(34.3%)	23(65.7%)	1.69(0.79–3.61)	1.63(0.55–4.81)	
	>1000 ETB	55(23.6%)	178(76.4%)	1	1	
ANC follow up	No	10(35.7%)	18(64.3%)	1.63(0.72–3.72)	0.35(0.05–2.69)	0.312
	Yes	66(25.4%)	194(74.6%)	1	1	
Iron and folic acid supplementation	No	19(39.6%)	29(60.4%)	**2.10(1.10–4.03)**	1.40(0.05–37.1)	0.842
	Yes	57(23.8%)	183(76.2%)	1	1	
Trimester iron or folic acid supplementation started	First trimester	14(16.7%)	70(83.3%)	1	1	0.503
	Second Trimester	34(26.6%)	94(73.4%)	1.81(0.90–3.62)	1.45(0.66–3.21)	
	Third trimester	9(32.1%)	19(67.9%)	2.37(0.89–6.30)	1.89(0.59–6.10)	
History of vaginal bleeding during pregnancy	Yes	11(37.9%)	18(62.1%)	1.82(0.82–4.06)	1.02(0.31–3.32)	0.977
	No	65(25.1%)	194(74.9%)	1	1	
History of intestinal parasite infection during pregnancy	Yes	17(35.4%)	31(64.6%)	1.68(0.87–3.26)	1.52(0.67–3.50)	0.320
	No	59(24.6%)	181(75.4%)	1	1	
History of Malaria infection during pregnancy	Yes	8(40%)	12(60%)	1.96(0.77–5.00)	1.90(0.54–6.69)	0.316
	No	68(25.4%)	200(74.6%)	1	1	
Meat or animal product consumption per week	No	17(37.8%)	28(62.2%)	1.89(0.97–3.70)	1.13(0.40–3.21)	0.817
	Yes	59(24.3%)	184(75.7%)	1	1	
Vegetable consumption per week	Yes	59(23.1%)	196(76.9%)	**0.28(0.14–0.60)**	**0.26(0.11–0.62)**	**0.003**
	No	17(51.5%)	16(48.5%)	1	1	

(*Continued*)

Table 4 (Continued).

Characteristics of Study Participants		Anemic Newborns	Non-anemic Newborns	COR (95%CI)	AOR (95%CI)	P-value
Fruit consumption per week	No	13(46.4%)	15(53.6%)	**2.71(1.22–6.00)**	0.51(0.10–2.56)	0.413
	Yes	63(24.2%)	197(75.8%)	1	1	
Nutritional status (MUAC)	Malnourished	12(36.4%)	21(63.6%)	1.71(0.80–3.67)	1.13(0.37–3.49)	0.829
	Normal	64(25.1%)	191(74.9%)	1	1	
Newborn birth weight	Low birth weight	10(30.3%)	23(69.7%)	1.25(0.56–2.75)	1.82(0.72–4.57)	0.203
	Normal	66(25.9%)	189(74.1%)	1	1	
Maternal anemia	Yes	23(39.7%)	35(60.3%)	1	1	**0.003**
	No	53(23%)	177(77%)	**0.46(0.25–0.84)**	**0.34(0.17–0.69)**	

Note: Bold numeric indicate significant association (p-value <0.05).
Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio.

participants in Iran study were born by cesarean section. The placental transfusion force and duration may be weak during a cesarean section. This could be because of the effect of anesthesia, the uterine incision, and the immediate clamping of the umbilical cord, as well as the lack of uterine or vaginal pressure, which forces fluids out of the fetus's lungs and supports breathing[39] and compared to a vaginal delivery, an inadvertent incision of the placenta causes hemorrhage and anemia.[11,36]

The possible difference between our study and Benin might be attributed to that the Benin study was conducted among most of the newborns delivered to malaria-infected mothers. However, only 6.9% of newborns born from mothers having a history of malaria were included in the current study. Due to placental barrier disruption or damage, malaria parasites enter the fetus through congenital and resulting in fetal immune activation in response to maternal malarial antigens and causing the fetal RBC to be destroyed and then this resulted in lower Hgb (anemia).[40]

Another possible reason for the difference between the current study and the Benin study could be the inclusion of preterm newborn, whereas the current study only included term newborns. Premature babies are not yet of full gestational age, most of the iron needed by the newborn is stored during the third (last) trimester. As a result, premature newborn have a negative iron balance, leading to the development of anemia.[41] Additionally, compared to full-term newborns, premature newborns' kidneys are still immature and cannot produce enough erythropoietin, leading to the development of anemia.[42]

The deviation of this study from the study conducted in Nigeria and Ghana could be due to all study newborns being born to HIV negativemothers. However, study participants born to HIV positive mothers were included in studies conducted in Ghana and Nigeria. In comparison to our study, HIV may increase the prevalence of anemia in newborns. According to a report from Kenya, having an HIV positive mother increases the risk of newborn anemia both directly through mother-to-child HIV transmission and indirectly through the fact that babies of HIV positive mothers have worse anemia than babies of HIV negative mothers.[43]

In the current study, based on red blood cell indices especially MCV value (normal range: 91.6–113.22),[2] microcytic, normocytic and macrocytic anemia among newborns were 7.9%, 31.6%, and 60.5% respectively. Additionally, 183 (76.2%) babies born to mothers taking iron and folic acid supplementation were non-anemic but, 57 (23.8%) babies born to mothers not taking iron and folic acid supplementation were anemic. The possible reason for those may be due to anemia in a newborn can be grouped into three major classes based on specific causes; which is anemia caused by blood loss, impaired RBC production, and increased destruction of RBC. Blood loss is the commonest cause of neonatal anemia including obstetrical causes (placental abruption, placenta previa, and trauma to placenta or umbilical cord during delivery), feto-maternal hemorrhage, fetoplacental transfusion, twin-twin transfusion, and internal hemorrhage. Increased destruction of RBC can happen as a result of immune hemolysis (blood group incompatibility), acquired hemolysis (like due to infection), and rarely hereditary RBC disorders including RBC enzyme defects, RBC membrane defects,

hemoglobinopathies, congenital hemolytic anemia (α-thalassemia, membrane disorders). Impaired RBC production can happen as a result of aplastic or hypoplastic anemia and Bone marrow suppression.[13,44]

Based on this finding, maternal dietary habit of eating vegetables during pregnancy was significantly associated with newborn anemia, which is supported by studies published in Qatar[16] and Gondar.[11] The results showed that babies born from mothers who ate vegetables were 74% less likely to develop anemia than babies born from mothers who ate no vegetables. This may be because of that vegetables are important sources of micronutrients such as non-heme iron and folic acid, which are used by the mother and her newborn to synthesize Hgb and new red blood cells. And also vegetarians (who consume vegetables at least once per week) generally have enough iron because they get a lot of vitamin C from their diet, which helps with non-heme iron absorption. A reduced development of anemia in the newborn is the result of the fetus's active parasitic uptake of iron from the mother's circulation, which it then transfers into the fetal circulation through the placenta for Hgb synthesis.[45]

The other variable that showed a significant association with newborn anemia was maternal anemia which is supported by a study reported in Nepal,[28] Benin,[37] Nigeria,[32] and Ghana.[23] Babies born to non-anemic mothers were 34% less likely to develop newborn anemia as compared to newborns born from anemic mothers. This could be because low levels of Hgb are a late symptom of iron deficiency, and the low levels of Hgb in these newborns could be a sign of low iron stores at birth. This suggests that a mother's anemia during childbirth affects the hemoglobin levels of her newborn.

Hence, this observation contradicted the common belief that the fetus continues to extract and receive iron to meet its needs even if the mother is anemic. However, in severe maternal anemia, the placental mechanism of iron transport from the mother to the fetus may be impaired and leading to decreased cord blood hemoglobin levels.[10,46] The relationship between maternal anemia and newborn anemia is a complex one, and there are many unanswered questions. To fully understand this relationship, more research using molecular biological tools is required.

Overall, this study yielded important insights about the prevalence and associated factors of anemia in newborns at JMC. The results of this study can be used by health professionals and policymakers to plan improvements at this age. Therefore, effective intervention packages need to reduce anemia among pregnant women through iron or folic acid supplementation, improve maternal dietary habit of vegetable consumption and properly evaluated, control and treated anemia during pregnancy in the study area may reduce anemia in pregnant women and their babies.

The major limitation of this study was serum ferritin concentration test was not performed, which suggests that iron deficiency may be the cause of anemia in newborns. Furthermore, due to the cross-sectional nature of the study design and the absence of an assessment of the occurrence of hemorrhage between the fetus and the mother prior to delivery, it is also unable to infer direct biological causality.

Conclusion and Recommendations

Overall, newborn anemia was a moderate public health problem in this study based on WHO cut of values. Maternal vegetable consumption habits and maternal anemia were the factors significantly associated with anemia. Efforts should be made to reduce its burden on a newborn. The prevention of maternal and newborn anemia was positively impacted by improving maternal nutritional status, particularly vegetable consumption during pregnancy. To prevent negative effects (like anemia) on a newborn, anemia during pregnancy should be appropriately assessed, controlled, and treated accordingly. Furthermore, large-scale longitudinal studies with larger samples are needed to identify the specific etiology and causes of neonatal anemia.

Abbreviations

AOR, Adjusted odds ratio; CBC, Complete blood count; g/ dl, Gram per deciliter; Hgb, Hemoglobin; HIV, Human immunodeficiency virus; JMC, Jimma Medical Center; LBW, Low birth weight; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; MUAC, Mid-Upper Arm Circumference; RBC, Red Blood Cell; RDW, Red Cell Distribution Width; WBC, White blood cell; WHO, World Health Organization.

Data Sharing Statement

All important data are within the manuscript but the datasets used during analysis are available from the corresponding author on reasonable request (in SPSS code).

Ethics approval and consent to participate

Ethical clearance was obtained from the institutional review board (IRB) of Jimma University, Institute of Health with letter protocol number IHRPGD928/20 and our research protocol meets ethical standards outlined by the declaration of Helsinki, national and international guidelines. After ethical approval was received, permission to conduct the study was obtained from the Head of the School of Medical Laboratory Science and the chief clinical director of the JMC.

Written informed consent was obtained from each mother for participation in this study after explaining the objective and purpose of the study. All methods include umbilical cord blood sample collection from newborns were performed in accordance with the relevant guidelines and regulations that meets national and international guidelines as approved by an appropriate ethics committee. Any abnormal hematological test results of study subject were communicated to their attending physician immediately proper management and treatment.

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Disclosure

The authors reported that they have no competing interests and no need of consent for publication.

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SHORT REPORT

The Montana Interfacility Blood Network: A Novel Lifesaving "Hand-off" for the Optimal Care of Rural Patients

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Purpose: The state of Montana encompasses and defines rural health care as it is known in the United States (US) today. This vast area is punctuated by pockets of health care availability with varying access to blood products for transfusion. Furthermore, timely transport is frequently challenged by weather that may limit air transportation options, resulting in multiple hours in ground transport to definitive care.

Patients and Methods: The Montana State Trauma Care Committee (MT-STCC) developed the Montana Interfacility Blood Network (MT-IBN) to ensure blood availability in geographically distanced cases where patients may otherwise not survive. The index case that led to the formal development of the MT-IBN is described, followed by a second case illustrating the IBN process.

Results: This process and development manuscript details the innovative efforts of MT-STCC to develop this fledgling idea unique to rural US health care. We review guidelines that have been developed to define broad aspects of the MT-IBN including the reason to share resources, proper packaging, paperwork necessary for transfer, and how to provide resources directly to the patient. Finally, we describe implementation within the state.

Conclusion: The MT-IBN was developed by MT-STCC to facilitate the hand-off of lifesaving blood to patients being transported by ground to definitive care in Montana without having to stop at an intermediary facility. This has already led to lives saved in areas that are limited in blood availability due to rurality.

Keywords: rural health care, blood transfusion, health services accessibility, trauma

Introduction

Background

Massive hemorrhage remains a major cause of preventable death in trauma.[1] Of these deaths, 33–56% occur during the prehospital period.[2] The key components of treatment are immediate hemorrhage control[3] and blood product transfusion to restore volume and manage coagulopathy.[4] The European guidelines on management of major bleeding and coagulopathy following major trauma[5] suggest that bleeding trauma patients should be rapidly transported to major trauma centers to institute appropriate treatment as soon as possible. Furthermore, the importance of immediate access to blood products for hemorrhagic shock is well known in the trauma literature. Hemorrhage is responsible for 30–40% of trauma mortality.[2]

The state of Montana (Figure 1A) encompasses over 147,000 square miles with approximately 7 people per square mile,[6] which truly defines rural health care as it is known in the United States today. This vast area is punctuated by pockets of health care availability with varying access to blood products for transfusion and surgical care. Furthermore, timely transport to definitive care is challenged by weather that may limit air transportation options, resulting in multiple hours in ground transport. Herein, we report the index case prompting the development of a novel interfacility blood network, and a second case illustrating the implementation and outcome of the new process.

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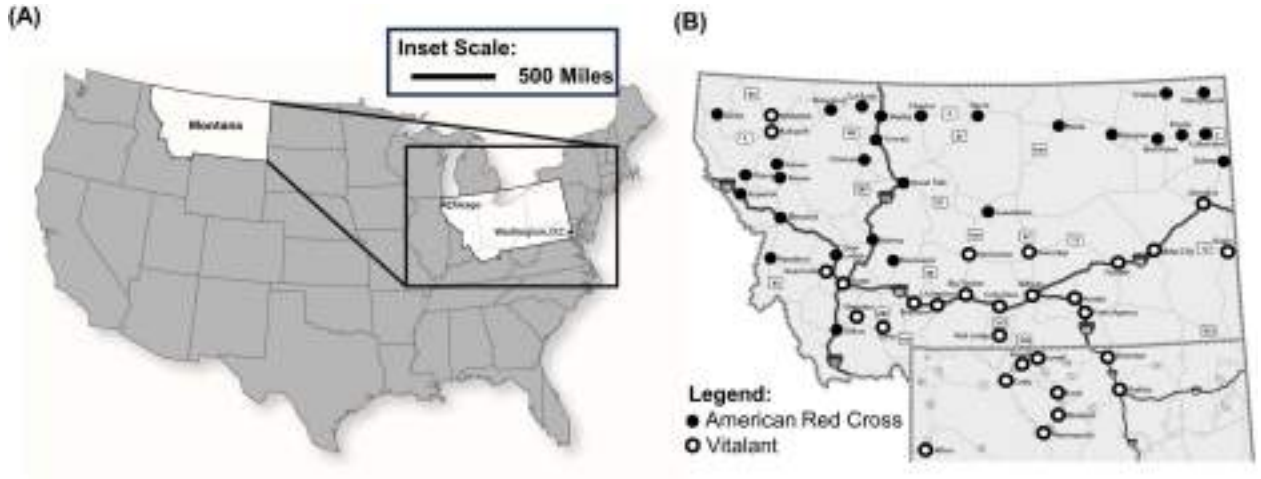

Figure 1 State of Montana Perspective. (**A**) A graphical representation of the United States with emphasis on the state of Montana scale. (**B**) A geographic display of packed red blood cells (PRBCs) locations throughout the states of Montana and northern Wyoming.

Index Case #1

A 30-year-old female presented to a rural health care center in Lame Deer, MT with severe abdominal pain and a syncopal episode. The patient developed hypotension with transient response to fluid resuscitation, with no blood product availability in this associated clinic or region. Due to the frigid weather, ongoing snowfall, and low aviation ceiling, helicopter and fixed wing transport were not possible. The patient was transported by ground 104 miles to the closest integrated tertiary care and Level II trauma center, in Billings, MT. Enroute, the patient required bag mask ventilation due to altered mental status 60 miles from Billings and then went into cardiac arrest 7 miles from Billings requiring CPR. Upon arrival at the tertiary facility, after a transfer time of 3 hours and 18 minutes, the patient underwent massive transfusion protocol and immediate surgical intervention where she was noted to have a ruptured ectopic pregnancy with evacuation of 5 liters of intraperitoneal blood. Post-operatively, the patient was extubated and made a full physiologic and neurologic recovery.

Out of necessity and for the optimal care of patients, the Montana State Trauma Care Committee (MT-STCC) developed the Montana Interfacility Blood Network (MT-IBN). We hypothesize that the MT-IBN will ultimately save lives in a state dominated by vast distances between health care facilities.

Materials and Methods

All massive transfusions were peer reviewed at an ACS verified level II trauma center (tertiary trauma facility (regional care facility)) by a Blood Utilization Committee, a multi-disciplinary monthly meeting formed in August 2015 and led by a physician chair. In review of the Index Case #1, it was noted that ground transport passed two facilities that had blood availability on the way to definitive care. Further discussion remarked on the published availability of a state "microbrew map" and thus raised the question of why a state blood map was not available.

Blood banking specialists developed a preliminary map detailing locations of packed red blood cells (PRBCs) throughout the states of Montana and northern Wyoming (Figure 1B). The preliminary idea of a blood sharing network was then discussed at the MT-STCC on May 19, 2019. A follow-up discussion occurred at the August 14, 2019, MT-STCC meeting, and a blood subcommittee was then created on November 13, 2019. This blood subcommittee met twice through 2019 and 2020 in which the blood map was reviewed, and a blood sharing procedure draft was developed.

Step 1 - Determine Interest

To gauge potential interest across the state and the potential for provider buy-in, a questionnaire was initially distributed via the state trauma forums to all trauma coordinators at hospitals affiliated with the Montana Trauma system in January 2020; this consisted of 41 state trauma designated hospitals. Subsequently, the results were compiled and reported at the February 12, 2020, MT-STCC meeting (Supplemental Materials 1).

Step 2 - Guideline Development

A multidisciplinary team set forth to develop guidelines for a blood sharing network utilizing the blood product availability map. Five broad categories (Figure 2) were determined to be necessary for patient safety, blood banking regulatory concerns, and statewide implementation, including a blood product availability map.[7]

1. Reason to share resource identified
2. Blood resources must be available
3. Proper packaging
4. Paperwork for transfer
5. Resupply and billing

Step 3 - Stakeholder Review

The MT-IBN concept (Figure 3) was formally presented at the Montana statewide American Society for Clinical Laboratory Science (ASCLS) and the regional Rimrock Trauma Conference in April 2021. The developers further discussed the concept at various Trauma Education Assessment & Management (TEAM) courses, the Montana equivalent of the American College of Surgeons' Rural Trauma Team Development Courses,[8] throughout 2021. An update was provided to the Montana Department of Public Health and Human Services with a Clinical Laboratory Improvement Amendment (CLIA) update in December 2021 that was sent to laboratory providers statewide. Discussions then continued with a presentation at the Montana Public Health Association conference in April 2022 and included

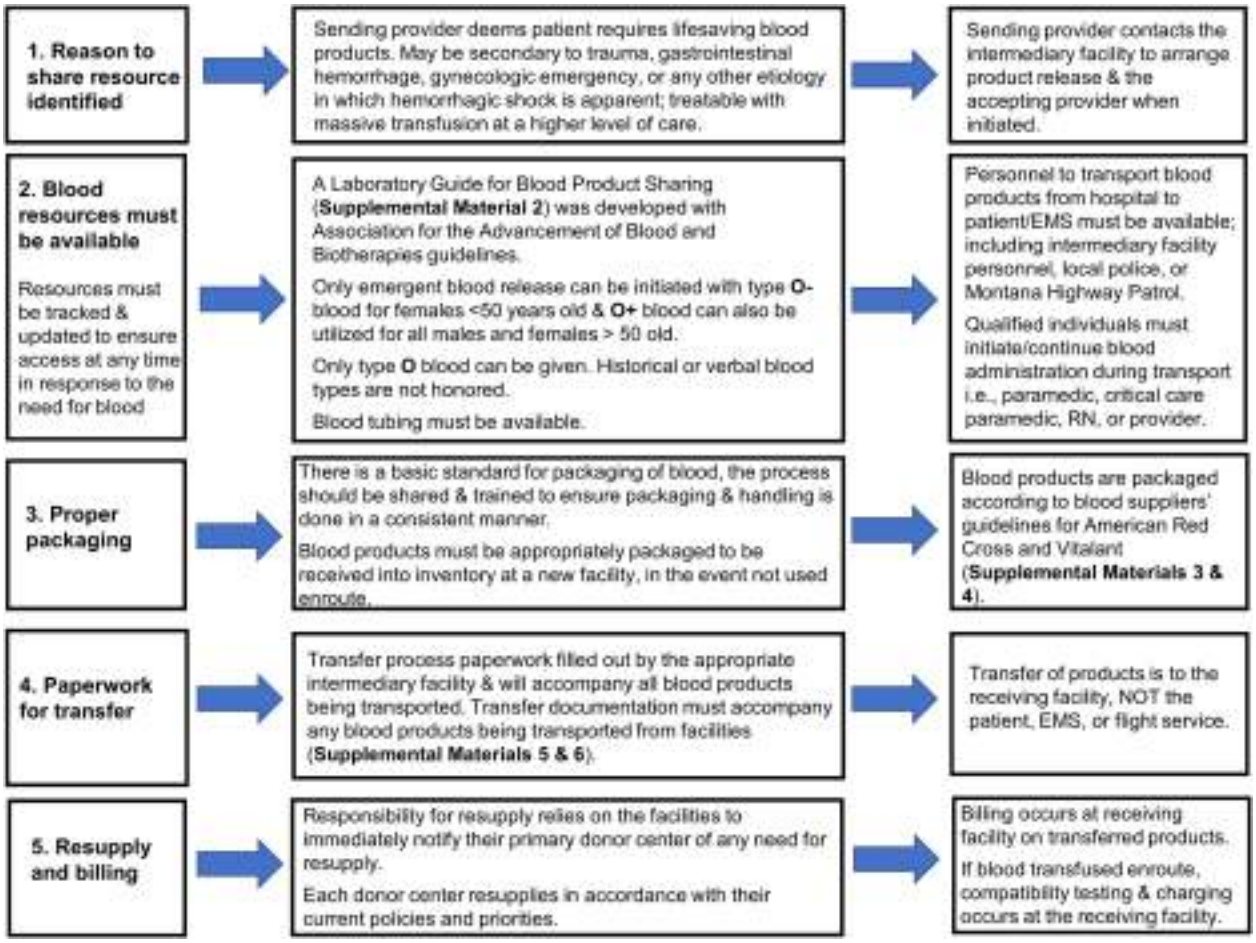

Figure 2 Blood Product Sharing Guidelines Developed for Statewide Implementation.
Note: Supplementary Materials can be accessed here: https://www.dovepress.com/get_supplementary_file.php?f=442134.docx.

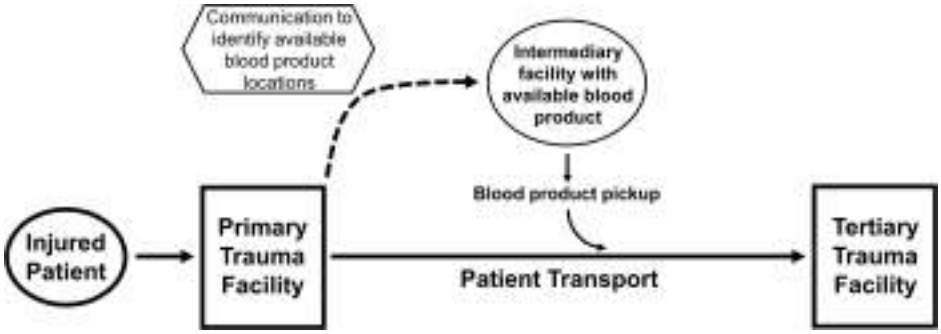

Figure 3 Diagrammatic Representation of the Montana Interfacility Blood Network Concept. Primary trauma facility refers to the first hospital facility a trauma patient arrives post-injury. Tertiary trauma facility refers to the highest level of appropriate trauma care for the patient.

meetings with key leaders of the Montana Hospital Association (MHA). Following this, the MT-IBN concept underwent final review and approval by MT-STCC in April 2022.

Step 4 – Implementation

Administrative policy and guidelines for personnel were distributed statewide along with education for Emergency Medical Services (EMS) and trauma personnel as discussed further in the Results.

Written informed consent was obtained from the patients for publication of their cases.

Results

The questionnaire was sent to all hospitals designated by the Montana Trauma system and included trauma coordinators, Trauma Medical Directors, and trauma staff (n=112), with the aim to obtain a respondent from each facility. A total of 40 individuals from 41 state-designated facilities responded to the questionnaire. Aproximately 92.5% responded that the ambulance should be able to pick up blood products for administration to a patient during transport to a higher level of care (Supplemental Materials 1). Furthermore, 75% of respondents believed that such a process was either probably or definitely needed. Aproximately 48.7% responded that sharing of blood products for patients "passing by" and not seen at the facility was allowed, but without policy or process. However, many responded that gaining approval for such process at their facility would be somewhat difficult (57.5%), very difficult (12.5%), or extremely difficult (5%).

Following approval of the MT-IBN at STCC, packets were compiled containing the following: a cover letter, a quick start guide containing the five broad guidelines discussed above, Laboratory Guide for Blood Product Sharing (Supplemental Materials 2), packing guidelines (Supplemental Materials 3 and 4), and transfer documents (Supplemental Materials 5 and 6). Rollout included respective education of EMS and trauma personnel at the Rocky Mountain Rural Trauma pre-conference symposium and of hospital providers at the MHA in September 2021.

Case #2

A patient with severe gastrointestinal (GI) hemorrhage presented to a rural critical access hospital (CAH) in rural Montana where workup revealed a hemoglobin of 5.8 gm/dl. This facility had only 1 unit of O negative blood available. The patient required ground transport from the primary CAH to definitive care in Billings (82.8 miles away). Midway between the rural facility and Billings on the Interstate Highway is another Critical Access Hospital, 41.3 miles from the primary CAH. The provider at the primary CAH called the nurse supervisor of the midway CAH at 0200 to discuss the situation and confirm if they could provide intermediary lifesaving blood. Laboratory personnel at the midway CAH were called in, readied a unit of O negative blood, and arranged for the local law enforcement officer to take the blood to the on/off ramp to the highway. The patient did not need to be registered, observed, or unnecessarily delayed at the midway CAH. Instead, the ambulance met the local law enforcement officer at the highway on/off ramp, and the ambulance crew transferred and transfused the required blood on the way to the Billings facility. The patient had a favorable outcome.

Discussion

Two recent randomized controlled trials, Prehospital Air Medical Plasma (PAMPer)[9] and the Control of Major Bleeding After Trauma Trial (COMBAT),[10] provided opposing views on the implementation of prehospital plasma transfusion. However, a post hoc analysis combining the two trials suggested that there was a benefit when prehospital transfer times are longer than 20 minutes.[11] Additionally, distance from a trauma center and time to treatment are well-documented sources of disparity in rural care. A 2019 study[12] demonstrated that states with poor trauma center access had relatively higher pre-hospital death rates. These states with a high pre-hospital death burden had a lower proportion of the population with access to a Level I/II trauma center within 1 hour of injury.[12] This is further supported by other studies which have demonstrated that rural residents are 14% more likely to die from traumatic injury compared to non-rural residents.[13]

Patient care in Montana is influenced by inherent rural disparities and geographic isolation (Figure 1A). Despite this land mass, the 2020 US census demonstrated an average population density of 7.4 people per square mile compared to the average US population of 94 per square mile. The Montana state trauma system was comprised of 41 state-designated trauma facilities (4 ACS-verified Level II, 3 ACS-verified Level III, 11 community trauma hospitals, and 23 trauma receiving facilities). Timely access to a trauma center is not possible in many areas of the state at the current time.

The majority (75%) of respondents to the questionnaire believed that the MT-IBN blood handoff was necessary for optimal patient outcomes but that it would be difficult gaining administrative approval at their individual facility. MT-STCC collaboration with numerous state societies in conjunction with MT-STCC oversight of state trauma destination protocols helped alleviate individual facility administrative difficulties and garner widespread adoption. This led to a profound impact on rural health care delivery with very preliminary evidence of improved survival in the state as seen in Case #2.

During initial MT-IBN discussions, the top concern from providers was to avoid a transfusion-based reaction. A decision was made early in development to begin implementation with PRBCs only and exclude fresh frozen plasma or platelets. Only type O blood was given, with type O negative for females less than 50 years of age; whilst type O positive blood was available for males (18 years of age and older) and females 50 years of age and older. Transfusion of emergent release PRBCs before completion of routine blood bank testing carries a low risk of non-ABO alloantibody mediated hemolytic transfusion reaction rate of 0.4%.[14] Other studies have demonstrated a 3% rate of alloimmunization due to emergent release transfusion, a 0.3% rate of incompatible, and a 0.02% rate of delayed hemolytic transfusion reaction.[15]

A secondary benefit of the MT-IBN includes enhanced care of non-trauma patients such as gynecologic/obstetric bleeding diathesis and GI hemorrhage. The development of the network ensures blood availability in cases whereby patients may otherwise not survive. The uniqueness of the MT-IBN stems from the fact that blood sharing may occur between dozens of unaffiliated hospital systems and small critical access facilities without the need for the patient to stop for care at the intermediary facility. The transport of patients in the MT-IBN does not represent an Emergency Medicine Treatment and Active Labor Act (EMTALA) violation, as patients are stopping at an intermediary facility for a resource instead of an evaluation.[16]

The closest published and known use of prehospital transfusion such as the MT-IBN concept is through the Southwest Texas Regional Advisory Council (STRAC). STRAC is a collaboration with South Texas Blood and Tissue Center, UT Health San Antonio, University Health Systems, and the US Army Institute of Surgical Research/San Antonio Military Medical Center. STRAC has developed a network of cold stored whole blood products which can be delivered and transfused in the prehospital setting on helicopters and by emergency medical services. They utilized a "Brothers in Arms" system which relies on a broad network of O positive males for donation.[17,18] The rurality of Montana would not make this process feasible, and thus the MT-IBN is better suited for this region of the country.

Limitations

The development of this project had limitations. Federal laws dictate that without the appropriate licensing, blood cannot cross state lines. Even though some tertiary centers in Montana have affiliates in Wyoming, Idaho, and the western Dakotas, blood cannot cross over state lines in transport on the way to definitive care. There are current ongoing regulatory discussions to reconcile this issue. Furthermore, participation in MT-IBN is not mandated, and thus, some facilities may opt out of participation if asked to act as an intermediary/donating facility.

Conclusion

The MT-IBN was developed by the MT-STCC to facilitate the hand-off of lifesaving blood to patients being transported by ground to definitive care in Montana. This unique, unparalleled network has already led to lives saved in a state with limited blood availability due to rurality.

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 in this work.

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ORIGINAL RESEARCH

Immuno-Hematological and Biochemical Changes in Patients with Tuberculosis in Dessie Comprehensive Specialized Hospital, Dessie, Ethiopia

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Background: Tuberculosis (TB) is a serious worldwide health issue, particularly in developing nations like Ethiopia. Patients with tuberculosis experience a range of hematological, immunological, and biochemical alterations. The purpose of this study was to evaluate immunological, hematological, and biochemical alterations of newly diagnosed TB patients at Dessie comprehensive specialized hospital, Dessie, Ethiopia.

Methods: A comparative, cross-sectional study was carried out to evaluate the immuno-hematological and biochemical changes in patients with tuberculosis at Dessie comprehensive specialized hospital from January to July 2018. One hundred sixty-four (164) newly diagnosed TB patients, and 80 apparently healthy controls were included consecutively. The variables were expressed in frequency, percentage, and mean ± SD. To compare mean ± SD of the groups or within the groups, we used an independent sample *t*-test. Statistical significance was defined as a P value less than 0.05.

Results: Male TB patients had significantly high mean absolute WBC count, neutrophil count, lymphocyte, platelet count, and systemic immune-inflammation compared with male healthy controls (P=0.001, P=0.011 P=0.021, P=0.001, and P=0.018, respectively). The mean platelet count of female TB patients was significantly higher than that of the female control group (P=0.015). However, mean RBC counts, Hgb, HCT, and MPV of TB patients were significantly lower than those of male ($p<0.001$) and female healthy controls (P=0.022, 0.015, and 0.001, respectively). The TB patients had developed anemia (23.8%), WBC abnormalities (29.3%), thrombocytosis (11.6%), and thrombocytopenia (9.8%). The cases had significantly higher mean alanine amino transferase, total bilirubin, and glucose level, but the mean total protein, alkaline phosphatase, and total cholesterol of cases were significantly lower than healthy control groups.

Conclusion: TB patients in this study showed significant alterations in a number of hematological and biochemical profiles. This indicates that hematological and biochemical profiles should be monitored and properly interpreted for the differential diagnosis of tuberculosis and evaluation of response to treatment.

Keywords: tuberculosis, pulmonary tuberculosis, immunological markers, hematological parameter, lipid profile, biochemical change

Introduction

Mycobacterium tuberculosis (Mtb) is the causative agent of an infectious disease Tuberculosis (TB). It mostly affects the lower respiratory system (pulmonary TB) and is characterized by a chronic productive cough, low-grade fever, night sweats, and weight loss, but can affect other sites as well, which is called extra pulmonary TB.[1]

TB continues to be a critical health issue globally. It affects millions of people annually and is one of the top causes of mortality along with human immunodeficiency virus (HIV). In 2021, about 10.6 million new TB cases emerged, and

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1.6 million lives were lost. With an estimated TB incidence rate of 126 per 100,000 people per year, Ethiopia stands as the world's 10th most TB-affected nations.[2]

Mtb primarily invades macrophages, key players in immunity, creating a conducive environment for itself. The host's defense against Mtb relies significantly on T cell-driven responses. Various CD4 T cell types, including Th1, Th2, Th17, and regulatory T cells (Tregs), work together or interfere one another to manage the disease.[3,4]

The protective role of the Mtb specific CD4 Th1 cell response is underscored by its capacity to produce cytokines, notably interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α). These cytokines are crucial for attracting and activating innate immune cells, including monocytes, macrophages, and granulocytes. Individuals with deficiencies in IFN-γ and interleukin-12 (IL-12; which promotes TH1 cell differentiation) were highly vulnerable to tuberculosis. Additionally, the link between reduced CD4 T cell numbers and increased tuberculosis risk in HIV-positive individuals highlights the role of these cells in defense. Tuberculosis can also result in reduced CD4 and CD8 lymphocyte levels in patients who are not HIV-positive.[3–5]

Normally, the CD8+ T cell reaction to Mtb is not as strong as that of CD4+ T cells. Nevertheless, CD8+ T cells have the ability to influence the function of phagocytes and release substances like granulysin, which can kill mycobacteria. Moreover, cytokines beyond IFN, such as TNF, play a pivotal role in forming granulomas, structured clusters of immune cells that control the pathogen.[4]

Cholesterol may play a significant role in the cellular immune response. A diet high in cholesterol can increase the sterilization of sputum culture in those suffering from pulmonary tuberculosis (PTB), while insufficient cholesterol levels could negatively impact the function of lymphocytes and macrophages, potentially worsening tuberculosis. Moreover, reduced total cholesterol, HDL, and LDL levels have been observed in individuals with PTB. The degree of smear positivity in PTB patients also exhibits a notable link with serum lipid levels, highlighting its utility in evaluating lipid dyslipidemia in these patients.[6–8]

Tuberculosis primarily targets the lungs but can also severely impact the hematopoietic system. It causes a range of hematological abnormalities such as elevated ESR, anemia, and increased lymphocytes and platelet counts.[9,10]

Studies have evidenced a significant association between the presence of acid-fast bacilli in sputum and the occurrence of hematological and biochemical alterations in patients with TB. A deeper comprehension of the immune response to mycobacterial infections has provided insight on the association with blood irregularities. Indicators such as immunological markers, hematological abnormalities, and biochemical alterations may aid physicians in the diagnosis of TB.[8,11] However, there is a scarcity of information regarding these indicators in TB patients in our country Ethiopia. To address this gap, this study was conducted to investigate the immunological, hematological, and biochemical alterations of newly diagnosed patients with tuberculosis at Dessie comprehensive specialized hospital in Dessie, Ethiopia.

Methods and Materials

Study Design, Setting, and Population

A comparative, cross-sectional study was carried out to evaluate the immuno-hematological and biochemical alterations between patients with tuberculosis and healthy controls at Dessie comprehensive specialized hospital from January to July 2018. The hospital is located in Dessie town, South Wollo zone of Amhara Regional State, in north-eastern Ethiopia. It is 401 km far from Addis Ababa, the capital city. It serves as a referral hospital for people living in Wollo zones and neighbouring regions.

One hundred and sixty-four newly diagnosed TB patients and 80 apparently healthy controls were included in the study consecutively. The sample size was calculated by taking the following considerations: Power = 80%, Confidence interval = 95%, Ratio = 2:1, Mean ± SD of White blood cells (WBC) for case = 8.48 ± 3.09, and Mean± SD of White blood cells for control = 6.75 ± 1.83. The Mean ± SD of WBCs is taken from a study conducted in Jimma.[12]

Individuals on anti-TB therapy, younger than 18 years, pregnant women, HIV-positive persons, and those with a chronic illness history, such as hepatitis, diabetes, or renal disease, were not included in the research.

Data Collection Procedure

Trained clinical nurses gathered socio-demographic and clinical data from the study participants through a pretested questionnaire and reviewing medical records.

To perform the complete blood count (CBC), CD4 count determination, and clinical chemistry analysis, about six milliliters of venous blood specimen was collected from each participant (both TB patients and apparently healthy controls) into a di potassium EDTA anticoagulant tube and plain tube, 3 mL each. Sysmex KX-21N automated hematological analyzer (Sysmex corporation Kobe, Japan) was utilized to determine complete blood counts. CD4 T cell count of the study participants was determined using BD FACS count analyzer (Becton Dickinson and Company, California, USA), and Dirui CS T240 auto-analyzer (Dirui Industrial Company) was used to perform biochemical analysis.

To produce quality laboratory results, all manufacturer instructions and standard operating procedures were closely adhered to in every test procedure. To ensure the instruments' precision and the results' accuracy, quality control substances were tested alongside the patient specimens, and reagent expiration dates were verified.

According to the World Health Organization guidelines, anemia was identified by hemoglobin (Hgb) levels: less than 13 g/dl for males aged 15 and older and less than 12 g/dl for non-pregnant women. Anemia is categorized as mild, moderate, and severe depending on its severity. Mild anemia is defined as having Hgb concentrations ranging from 11.0 to 12.9 g/dl for men and from 11.0 to 11.9 g/dl for non-pregnant women. Moderate anemia is defined as hemoglobin level between 8.0 and 10.9 g/dl and severe anemia diagnosed when Hgb fell below 8.0 g/dl for both sexes.[13]

Total WBC > $10.6 \times 10^3/\mu L$ and WBC < $3.6 \times 10^3/\mu L$ were used to define leukocytosis and leukopenia, respectively. On the other hand, total platelet count > $450 \times 10^3/\mu L$ and platelet count < $150 \times 10^3/\mu L$ were used to define thrombocytosis and thrombocytopenia, respectively.[14]

The absolute counts of neutrophils, lymphocytes, monocytes, and platelets were used to calculate neutrophil-to-lymphocyte (NLR), platelet-to-lymphocyte (PLR), monocyte-to-lymphocyte (MLR) ratios and systemic immune-inflammation index (SII) using the following equations: NLR = Neutrophils/Lymphocytes, MLR = Monocytes/Lymphocytes, PLR = Platelets/Lymphocytes, and SII = Platelets × Neutrophils/Lymphocytes.

Data Analysis

Data from the laboratory investigation and questionnaire were entered in to "EpiInfo version 3.1" and then exported to the statistical analysis program SPSS version 20.0 (Statistical Package for the Social Science). The Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to check the normality distribution of continuous variables. Percentages and frequencies were used to report categorical variables, and mean ± standard deviation (SD) was used to express continuous variables. To compare mean ± SD of the groups or within the groups, we used Student's *t*-test (independent sample *t*-test). Statistical significance was defined as a P value less than 0.05.

Ethical Consideration

Ethical Review Board of College of Medicine and Health Science, Wollo University approved the study. The study was conducted according to the principles stated in the Declaration of Helsinki. All the study subjects (cases and controls) were 18 years and above and briefed about the study's objective and included after obtaining written consent. Participants' data was coded to maintain confidentiality, and attending doctors were informed of any unusual findings.

Result

Socio-Demographic Characteristics of the Study Participants

In this study, 80 healthy controls and 164 newly diagnosed TB patients were included. There were 93 (56.7%) males and 71 (43.3%) females among the cases, and 43 (53.8%) males and 37 (46.2%) females among the health controls. The sex distribution between TB patients and healthy controls did not differ statistically significantly (p=0.765). Majority of the TB patients were aged between 25 and 34 years 57 (34.8%), had no formal education 70 (42.7), were married 117 (71.3%), and had pulmonary type of tuberculosis 139 (84.8%) (Table 1).

Table 1 Distribution of Age, Sex, Educational Status, Marital Status, Tuberculosis Type, and HIV Status of Newly Diagnosed TB Patients at Dessie Referral Hospital, Dessie, Ethiopia (n=164)

Variable	Category	Frequency (n)	Percent (%)
Age in years	15–24 25–34 35–44 45–54 ≥55	19 57 51 24 13	11.6 34.8 31.1 14.6 7.9
Sex	Male Female	93 71	56.7 43.3
Educational Status	No education Primary Secondary Tertiary	70 33 46 15	42.7 20.1 28.0 9.1
Marital status	Single Married Divorced	33 117 14	20.1 71.3 8.5
Tuberculosis type	Pulmonary Extra	139 25	84.8 15.2

Immuno-Hematological Characteristics Between Healthy Controls and Newly Diagnosed Tuberculosis Patients

Although the average CD4 count of patients with pulmonary TB (565.04 ± 325.49) was lower than that with extra TB (672.88 ± 292.73), the difference was not statistically significant (P=0.124).

Male TB patients had significantly increased average absolute WBC count (7.79 ±3.71 × 10^3 cells/μL), neutrophil count (4.08 ± 2.74 × 10^3 cells/μL), lymphocyte (2.83 ±1.90 × 10^3 cells/μL), platelet count (320.66 ±156.58 × 10^3 cells/μL), and systemic immune-inflammation (SII) (702.85 ± 1382.55) compared with male healthy control's mean absolute WBC count (6.05 ± 1.99 × 10^3 cells/μL; P=0.001), neutrophil count (3.08 ±1.74 × 10^3 cells/μL; P=0.011), lymphocyte count (2.31 ± 0.65 × 10^3 cells/μL; P=0.021), platelet count (236.21 ± 59.73 × 10^3 cells/μL; P=0.001), and systemic immune-inflammation (SII) (345.49 ± 251.40, P=0.018). Compared with female healthy controls, female TB patients had elevated average absolute WBC count, neutrophil count, lymphocyte count, and SII, but the difference was not statistically significant (P=0.139, 0.673, 0.092, and 0.748 respectively). The average platelet count of female TB patients was significantly higher than the female control group (P=0.015) (Table 2).

Mean RBC counts, Hgb, and HCT of TB patients were significantly lower than those male (p<0.001) and female healthy controls (P=0.022, 0.015, and 0.001 respectively). Red cell distribution width (RDW) was significantly higher in female TB patients than female controls (p<0.001). Mean platelet volume (MPV) was significantly decreased in patients with tuberculosis compared to controls (p=0.001) (Table 2).

Hematological Abnormalities of TB Patients

Of the TB patients, about 23.8% (39) had anemia. Mild, moderate, and severe anemia were present in 79.5%, 17.94%, and 2.56% of the anemic patients, respectively. Normocytic anemia 20 (51.3%) was the most prevalent type of anemia, followed by microcytic anemia 18 (46.2%). The prevalence of anemia was higher in pulmonary TB patients 24.5% (34) than extra pulmonary TB patients 20.0% (5) (Table 3).

WBC abnormalities were present in 29.3% (48) TB patients: 19.5% (32) of the TB patients had leukocytosis and 9.8% (16) had leukopenia. Neutrophilia, neutropenia, lymphocytosis, lymphocytopenia, monocytosis, and

Table 2 Comparison of Average Values of Hematological Parameters in Healthy Controls and Newly Diagnosed TB Patients

Hematological Parameters	Male			Female		
	TB Patient Mean ± SD	HC Mean ±SD	P-value	TB Patient Mean ±SD	HC Mean ±SD	P-value
WBC × 10^3 cells/μL	7.79 ±3.71	6.05±1.99	0.001	8.21 ± 5.41	6.80 ± 2.60	0.139
Neu × 10^3 cells/μL	4.08 ± 2.74	3.08 ±1.74	0.011	4.12 ± 2.84	3.89 ± 2.46	673
Lym × 10^3 cells/μL	2.83 ±1.90	2.31 ±0.65	0.021	3.19 ± 4.16	2.33 ± 0.77	0.092
Mon × 10^3 cells/μL	0.66 ±1.00	0.51 ±.28	0.333	0.69 ± 0.66	0.47 ± 0.20	0.012
Eos × 10^3 cells/μL	0.17 ±.54	0.12 ±.14	0.522	0.13 ± 0.14	0.08 ± 0.08	0.053
Baso × 10^3 cells/μL	0.04 ±.05	0.03 ±.02	0.048	0.07 ± 0.14	0.03 ±.02	0.020
RBC× 10^6cells/μL	4.75 ±.68	5.51 ±.62	0.000	4.56 ±.75	4.83 ± 0.457	0.022
Hgb g/dl	14.19 ± 2.59	15.55 ±1.47	0.000	13.36 ±1.79	14.09 ± 1.23	0.015
HCT %	40.99 ± 6.42	47.84 ±4.95	0.000	38.65 ± 5.23	42.19 ± 4.45	0.001
MCV fl	86.24 ± 11.47	85.34 ±10.20	0.661	84.33 ± 9.38	87.64 ± 4.70	0.016
MCH Pg	30.28 ±4.81	28.25 ±1.71	0.000	29.62 ± 4.18	30.35 ± 3.84	0.590
MCHC g/dl	35.43 ± 3.85	32.48 ±1.09	0.000	34.93 ± 2.23	32.70 ± 0.69	0.000
PLT × 10^3 cells/μL	320.66 ±156.58	236.21 ±59.73	0.001	311.47± 124.22	266.78 ± 63.31	0.015
RDW SD	43.57 ± 3.50	44.48 ±3.15	0.133	43.06 ± 4.10	43.13±2.99	0.925
RDW CV	12.99 ±.81	13.03 ±1.63	0.831	13.19 ± 0.99	12.69 ± 0.43	0.000
PDW	15.73 ± 2.61	16.45 ± 2.15	0.121	15.91 ± 2.76	16.04 ± 2.02	0.808
MPV	9.43 ±1.27	10.20 ±1.08	0.001	9.38 ± 1.31	10.03 ± 0.98	0.010
NLR	2.08 ± 2.99	1.45 ± 0.98	0.181	2.04 ± 2.21	2.06 ±2.98	0.966
PLR	178.41 ± 408.45	109.42 ± 41.83	0.272	133.69 ± 73.81	125.34 ± 49.24	0.537
MLR	0.248 ± 0.25	0.23 ± 0.13	0.675	0.28 ±0.22	0.23 ± 0.14	0.234
SII	702.85 ± 1382.55	345.49 ± 251.40	0.018	571.22 ± 660.52	527.05 ± 707.23	0.748

Abbreviations: HC, Health control; Neu, Neutrophil; Lym, Lymphocyte; Mon, Monocyte; Eos, Eosinophils; Baso, Basophile; NLR, Neutrophil lymphocyte ratio; PLR, Platelet lymphocyte ratio; MLR, Monocyte lymphocyte ratio; SII, systemic immune-inflammation.

Table 3 Hematological Abnormalities of TB Patients

Hematological Parameters	Category	Frequency (%)
Hemoglobin	Anemic Non-anemic	39 (23.8) 125 (76.2)
Anemia severity (n=39)	Mild Moderate Severe	31 (79.50) 7 (17.94) 1 (2.56)
Mean red blood cell volume	Macrocytic Normocytic Microcytic	12 (7.3) 117 (71.3) 35 (21.3)
Platelet count	Thrombocytosis Normal thrombocyte Thrombocytopenia	19 (11.6) 129 (78.7) 16 (9.8)
Mean platelet volume	Macrothrombocytes Normal Microthrombocytes	4 (2.4) 158 (96.3) 2 (1.2)
White blood cell Count	Leukocytosis Normal Leukopenia	32 (19.5) 116 (70.7) 16 (9.8)

(*Continued*)

Table 3 (Continued).

Hematological Parameters	Category	Frequency (%)
Neutrophil count	Neutrophilia Normal Neutropenia	20 (12.2) 119 (72.6) 25 (15.2)
Lymphocyte count	Lymphocytosis Normal Lymphocytopenia	38 (23.2) 121 (73.8) 5 (3.0)
Monocyte	Monocytosis Normal Monocytopenia	13 (7.9) 128 (78.0) 23 (14.0)

monocytopenia were present in 12.2% (20), 15.2% (25), 23.2% (38), 3 0.0% (5), 7.9% (13), and 14.0% (23) of the patients, respectively (Table 3).

The prevalence of thrombocytosis and thrombocytopenia among the TB patients were 11.6% (19) and 9.8% (16), respectively (Table 3).

Biochemical Parameters

TB patients had significantly high mean Alanine amino transferase (30.70 ± 29.17), total bilirubin (0.839 ± 1.10), and glucose level (119.14 ±59.91) compared with healthy control's mean Alanine amino transferase (24.91 ± 10.77, P=0.026), total bilirubin (0.538 ±.39, P=0.010), and glucose level (101.35 ± 52.35, P=0.039). However, the mean total protein (6.37 ± 1.26), Alkaline phosphatase (166.23 ±118.49), and total cholesterol (159.56 ±45.43) of TB patients were significantly lower than healthy control's mean total protein (7.36 ±.61, P<0.001), Alkaline phosphatase (215.34 ± 101.35; P=0.002), and total cholesterol (192.95 ± 61.996; P=0.002) (Table 4).

Discussion

Tuberculosis remains a significant global infectious disease and a primary health concern in Ethiopia. It causes a range of hematological, immunological, and biochemical alterations.[2,8,11] The objective of the study presented here was to investigate these changes in newly diagnosed tuberculosis patients at Dessie comprehensive specialized hospital in Dessie, Ethiopia.

Table 4 Comparison of Mean Values of Biochemical Parameters in the Newly Diagnosed TB Patients versus Healthy Control

	TB Patient Mean ± SD	HC Mean ±SD	P-value
Alanine amino transferase (IU/L)	30.70 ± 29.17	24.91 ± 10.77	0.026
Aspartate amino transferase (IU/L)	39.48 ± 46.4	36.93 ±17.98	0.636
Total protein	6.37 ± 1.26	7.36 ±.61	0.000
Total bilirubin (mg/dL)	0.839 ± 1.10	0.538 ±.39	0.010
Alkaline phosphatase (IU/L)	166.23 ±118.49	215.34 ± 101.35	0.002
Blood urea nitrogen (mg/dL)	12.04 ±7.27	11.57 ± 3.62	0.525
Creatinine (mg/dL)	0.85 ± 0.60	0.86 ±.17	0.845
UREA	25.71 ± 14.62	24.36 ±7.64	0.356
Glucose (mg/dL)	119.14 ±59.91	101.35 ± 52.35	0.039
Triglycerides	133.93 ± 66.01	127.38 ±82.91	0.665
Total cholesterol	159.56 ±45.43	192.95 ± 61.996	0.002

Abbreviation: HC, Health control.

This study showed that Male with tuberculosis exhibited significantly elevated average levels of white blood cells, neutrophils, and lymphocytes in comparison to healthy male subjects. This result aligns with previous research from South Eastern Nigeria,[15] Saudi Arabia,[16] and Pakistan.[12] The immune system reaction to tuberculosis may be the cause of the rise in WBC count, neutrophil count, and lymphocyte count in TB patients. Neutrophilia is a sign of repeated continuous inflammatory reaction and this frequently transforms into lymphocytosis when the inflammatory response becomes chronic. However, our finding contradicts with another study in Kirkuk city.[17]

The average number of platelets in both male and female patients with tuberculosis was significantly elevated compared to the control group, and this is consistent with studies conducted in Iraq,[18] Kirkuk city,[17] Sudan,[19] South Eastern Nigeria,[15] Guyana,[20] and Jimma University.[12] These findings could be explained by reactive thrombocytosis, which can occur in a variety of clinical conditions including infections like pulmonary TB. Platelets, as key players in inflammation and immune responses, release pro-inflammatory cytokines such as IL-6 and TNF, which promote the synthesis of acute-phase proteins and thrombocytosis.[21,22]

The present study indicated that average RBC counts, Hgb level, and HCT of TB patients were considerably lower than the healthy controls group, for both sex. This agrees with Al-muhammadi et al,[18] Amilo et al,[15] Al-Omar et al,[16] and Jimma.[12] Reduced red blood cell life span, impaired marrow response, or poor iron transport from macrophages to the plasma in iron cycle metabolism, and chronic inflammatory disorder could be the cause of the decline in RBC number, Hgb, and HCT in TB patients.[23]

In this study, anemia had developed in around 23.8% (39) of the TB patients. It was more common in patient with pulmonary TB (24.5%) than in extra pulmonary TB (20.0%). There was mild-type anemia in the majority of the anemic patients. This finding is in line with a research done at St. Paul's Hospital Millennium Medical College (25%).[24] However, the prevalence of anemia is lower than other studies conducted in Jimma (37%),[12] Gondar (46%),[25] and India (77.3%).[26] Study participants' dietary habits, malabsorption syndrome, and the disease stage at diagnosis could be the contributing factors to the observed disparities. The most common type of anemia was normocytic anemia 20 (51.3%) followed by microcytic anemia 18 (46.2%) which is similar with studies conducted in Jimma University Specialized Hospital[12] and Seoul National University Hospital.[27]

The mean total cholesterol of the TB patients in this study was considerably lower than that of the healthy controls group. Reports from Casimir et al,[28] Taparia et al,[8] and Şahin and Yildiz[29] showed similar outcomes. A low cholesterol level makes one more vulnerable to different diseases like TB. A diet high in cholesterol has been found to hasten the process of bacteriologic sterilization in tuberculosis patients.[6,30] Total proteins were also significantly lower in patients with TB than in the healthy control group, which is similar to studies conducted by Şahin and Yildiz[29] and Modawe et al,[31] but the difference was not significant.

The mean Alanine amino transferase was significantly higher in TB patients than in the healthy control group, and this is consistent with the research done in Guyana.[20] However, the finding contradicted with the finding of Modawe et al on Biochemical Parameters in Relation to Tuberculosis in Sudanese Patients, which showed significantly lower alanine amino transferase in TB patients.[31]

The current study showed that TB patients had significantly higher glucose level than the control group, which is in line with the findings of a study by Bailey and Grant.[32] However, Kurup et al stated that blood sugar level did not show any variation between TB patients and the healthy control group.[20]

In conclusion, patients with tuberculosis had significantly higher mean absolute count of WBC, neutrophils, lymphocytes, and platelets compared to healthy controls. However, mean RBC counts, Hgb, HCT, and MPV of TB patients were significantly lower compared to healthy controls. TB patients had significantly higher mean Alanine amino transferase, total bilirubin, and glucose levels, but the mean total protein, alkaline phosphatase, and total cholesterol of TB patients were significantly lower than healthy control groups. TB has a significant impact on hematological and biochemical profiles. Therefore, the patients infected with TB should have their hematological and biochemical parameters monitored and properly interpreted for the differential diagnosis of tuberculosis and evaluation of response to treatment. Additionally, more studies should be conducted on TB and its effect on biochemical and hematological values in humans in the study area or other areas in the country.

Abbreviations

C, Cholesterol; CBC, Complete blood count; ESR, Erythrocyte Sedimentation Rate; Hgb, Hemoglobin; HIV, Human immunodeficiency virus; IFN-γ, Interferon gamma; MPV, Mean platelet volume; Mtb, Mycobacterium tuberculosis; PTB, Pulmonary tuberculosis; RBC, Red blood cell; RDW, Red cell distribution width; TB, Tuberculosis; WBC, White blood Cell.

Data Sharing Statement

All relevant data are included in the document.

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Author Contributions

All authors significantly contributed to the manuscript 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.

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Disclosure

The authors state that they have no competing interests.

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ORIGINAL RESEARCH

Hematological Abnormalities and Associated Factors Among Patients with Hypothyroidism at the University of Gondar Comprehensive Specialized Hospital

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Objective: Abnormalities in blood cells are frequently associated with thyroid hormone disorders as a result of their involvement in the proliferation and production of blood cells. This study aimed to determine the magnitude and associated factors of hematological abnormalities in patients with hypothyroidism.

Methods: A cross-sectional study was conducted from January 1 to June 30, 2023, at the University of Gondar Comprehensive Specialized Hospital. The present study included a total of 300 patients with hypothyroidism prospectively using the systematic random sampling technique. The hematological parameter data were collected using data extraction sheets, whereas the associated factor data were collected using both structured questionnaires and data extraction sheets. For complete blood cell counts, 4 mL of anticoagulated venous blood was collected and analyzed. The data were entered into Epi-data version 3.1 and analyzed with Stata version 14. Both bivariate and multivariate logistic regressions were performed to identify factors associated with hematological abnormalities. A P value < 0.05 was considered to indicate statistical significance.

Results: The median value of red blood cell, hemoglobin, mean cell volume, white blood cell, and platelet were 4.63 x10^{12}/μL, 14 g/dL, 84.3fl, 5.3 x10^{3}/μL, and 228, respectively. The overall incidences of anemia, leucopoenia, and thrombocytopenia in patients with hypothyroidism were 26.3% (95% confidence interval (CI): 21–32), 15.7% (95% CI: 14.2–17.2), and 9% (95% CI: 7.5–10.5), respectively. Lymphopenia was detected in 9% (95% CI: 8.6–10.1) of the patients, and neutropenia was detected in 6% (95% CI: 4.4–7.6) of the patients. Only three factors, female sex (adjusted odds ratio (AOR) =2.1, 95% CI=1.3–3.1), alcohol consumption (AOR= 3.8, CI=1.7–8.9), and febrile illness (AOR=2.7, 95% CI=1.3–5.4), were found to be significantly associated factors for anemia.

Conclusion: The present study revealed heterogeneous hematological abnormalities in patients with hypothyroidism. Thus, early diagnosis and monitoring strategies are required to minimize complications in patients.

Keywords: hematological abnormalities, anemia, leucopenia, thrombocytopenia, thyroid dysfunction

Introduction

Thyroid hormones are produced by the thyroid gland and are found in front of the neck.[1] It commonly results in dysfunction in individuals with endocrine disorders. It affects approximately 300 million people worldwide, with rates ranging from 4–10%, across different geographic locations.[2] Dysfunction is more prevalent in women than in men.[3] Due to thyroid hormone dysfunction, hypothyroidism occurs in 4.1% of 1000 women and 0.6% of 1000 males annually.[4,5] Similarly, thyroid

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dysfunction is noted in approximately 18% of the elderly population, but more than half of the population knows little about this condition.[6]

The thyroid gland synthesizes hormones such as triiodothyronine (T3) and thyroxine (T4). These hormones play a crucial role in regulating metabolic processes,[7] modulating metabolism, and hematopoiesis.[8,9] During hematopoiesis, thyroid hormones regulate the cell cycle, differentiation, and proliferation of erythrocytes, leucocytes, and platelets.[10]

Thyroid hormones regulate erythropoiesis through various mechanisms. It involves stimulating the production of erythropoietin, erythroid colony-forming units, and erythroid burst-forming units that induce erythropoiesis.[11,12] Besides, hormones regulate the signal transduction pathways involved in erythropoiesis. Furthermore, hormones are involved in balancing iron, vitamin B12, and 2.3-diphosphoglycerate levels during erythropoiesis.[4] With regard to leucopoiesis, this hormone regulates leukopoiesis by increasing the synthesis of granulocyte-monocyte colony-forming units and interleukin 3.[11,13] Evidence shows that thyroid hormone is not directly involved in thrombocytopoiesis but that increased concentrations of this hormone minimize the life span of thrombocytes.[8]

Dysfunction of thyroid hormones (hyperthyroidism and hypothyroidism) affects the production, differentiation, function, and survival of almost all blood cells.[8] Hypothyroidism is a chronic disease in which the thyroid gland produces a lower amount of thyroid hormone than needed in the body.[14] Hematological abnormalities such as anemia, leucopoenia, and thrombocytopenia are frequently observed in patients with hypothyroidism.[15] However, the magnitude of hematological abnormalities and risk factors for hypothyroidism vary from place to place.[16] Moreover, the associations between thyroid hormone levels and platelet count and mean platelet volume (MPV) are inconsistent.[17,18] Despite the fact that hypothyroidism has a significant effect on hematological parameters, there is variability in its magnitude and risk factors from place to place. In Ethiopia, only a limited number of studies have been performed. Even the reported study did not reveal all the hematological abnormalities. Furthermore, the associated factors were not thoroughly examined. Thus, the main objective of this study was to assess hematological abnormalities and associated factors among patients with hypothyroidism for early diagnosis of these abnormalities to decrease morbidity and mortality at the University of Gondar Comprehensive Specialized Hospital, Northwest, Ethiopia.

Methods and Materials

Study Design and Period

A hospital-based cross-sectional study was conducted from January 1 to June 30, 2023.

Study Area

The study was performed at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. The hospital is located in the central Gondar zone, Gondar town, Amhara Regional State. The town has an elevation of 2133 meters above sea level. The hospital provides health care to more than seven million people, including residents of Gondar town and neighboring zones. According to the data, the hospital provides services for approximately 1700 thyroid dysfunction patients annually.[19]

Populations

All patients with hypothyroidism who attended the University of Gondar Comprehensive Specialized Hospital were used as source populations in this study. Meanwhile, the study used patients with hypothyroidism who attended the study area during the data collection period and who fulfilled the inclusion criteria as a study population.

Variables

The dependent variable of the study was hematological abnormalities, whereas the independent variables were socio-demographic characteristics (age, sex, marital status, residence, educational status, and occupation), behavioral characteristics (smoking status, alcoholism, meat consumption, vegetable consumption, and iodine salt, consumption), and clinical characteristics (parasitic infection and febrile illness).

Eligibility Criteria

The current study included a total of 300 patients who consecutively visited the hospital during the data collection period and were newly diagnosed with hypothyroid disease by a physician. Patients with known hematological disorders; who were taking nonsteroidal anti-inflammatory drugs, phenazopyridine, or penicillin, who were pregnant, who recently received blood transfusions, who had surgery within the last three months and who had active traumatic bleeding were excluded from the present study.

Sample Size Determination and Sampling Technique

The sample size was calculated based on a single population proportion formula: $n= (Z\ \alpha/2)^2\ p\ (1-p)/d^2$ using 95% CI, $Z\alpha/2=1.96$, margin of error (d) 5%, and prevalence 26.5%, which was reported from a similar previous study. Consequently, the sample size of this study was calculated as $(1.96)^2 \times 0.265\ (1–0.265)/(0.05)^{2,}$ and 300 participants were ultimately included. Study participants included in this study were recruited using a systematic random sampling technique. Participants were selected based on two regular intervals (K).

Data Collection and Laboratory Methods

During the data collection period, two trained nurse professionals collected sociodemographic and behavioral characteristic data from the participants via a structured questionnaire and face-to-face interview. Moreover, the professionals collected the clinical data of the participants via an observation using data extraction sheet. In addition, complete blood count (CBC) data, stool examination data, and blood film data were collected after analysis using data extraction sheets by two trained laboratory professionals.

For CBC analysis, four milliliters of venous blood were collected by two trained laboratory professionals aseptically using a 19-gauge syringe. After collection, the blood was transferred to dipotassium ethylene diamine-tetraacetic acid (K_2EDTA) tubes, the tube was labeled with a unique identification number. To avoid blood clotting, the blood was mixed with EDTA anticoagulant gently. The collected whole blood was analyzed within 4 hours of being collected using a Beckman Coulter UniCel DxH 800 (Beckman Coulter, United States) automated hematology analyzer. The analyzer works based on the Coulter principle, VCS (volume, conductivity, and light scatter) technique, and spectrophotometry principle. During the analysis of the blood samples, the manufacturer's instructions were followed strictly. After analysis, the outputs of the analyzer were recorded in the data extraction sheets.

Current study included all patients who confirmed as having hypothyroidism by physicians. As a result, the result of thyroid function test was collected from patients medical chart. In the study setting, for thyroid function test, over night fasting venous blood was collected by laboratory professionals. All blood samples used for thyroid function analysis were collected by using plain tubes and allowed for 30 minutes at room temperature for clot formation. Then the serum was separated by centrifugation for 15 min at 2500 rpm using a centrifuge. After separation of serum, thyroid hormone level was measured using chemiluminescence immunoassay analyzer. The analyzer works based on the principle of immunoreaction. The binding of antigen and antibody result's immunoreaction. Subsequent addition of substrate in immune reaction results formation of light the intensity. The intensity of light formed is directly proportional to the amount of thyroid hormone level. Approximately 1 g of fresh stool sample from each respondent was collected by two trained laboratory professionals in a labeled, dry, clean, and leakproof container. After the collection of stool samples, the professionals prepared a wet mount and examined the parasites microscopically; the results were subsequently recorded on data extraction sheet. Similar professionals who examined stool samples prepared both thin and thick blood films for the detection of hemoparasite. The smears were prepared using a drop of anticoagulated venous blood and stained with a 10% Giemsa stain. Finally, the hemoparasite were examined and recorded on a data extraction sheet.

Data Quality Control

For data quality, the questionnaires were initially prepared in the English language, translated to the local language, and subsequently converted to the English language. These questionnaires were pretested at Tibebe Gihon Hospital Bahir Dar, Ethiopia, to assure consistency and validity. In addition, training was given to the data collectors to assure the quality of the data. Moreover, the data quality was assured by close inspection of the data collection process on site by

the principal investigators and supervisor to ensure its clarity, accuracy, completeness, and consistency. Furthermore, standard operating procedures were followed strictly during CBC analysis, stool examinations, and blood film examinations to assure the quality of the laboratory results.

Data Analysis and Interpretation

After the data were manually checked for completeness, the data were coded, entered into Epidata version 3.1, and subsequently analyzed via Stata version 14. Descriptive statistics such as frequencies and percentages were employed to summarize the data. Tables and charts used to present descriptive data. The presence of statistical associations between variables was assessed by chi-square and Fisher's exact tests. Moreover, bivariate and multivariate logistic regression models were used to evaluate the associations between hematological abnormalities and hypothyroidism in patients. The strength of associations was assessed by the crude odds ratio (COR) and AOR with 95% CI. A P value less than .05 was considered to indicate statistical significance. Assumptions such as the Shapiro–Wilk test were used to test the normality of continuous variables, whereas the Hosmer–Lemeshow test was used to check the model's fitness.

Operational Definitions

As defined by the World Health Organization (WHO), anemia is a condition in which the hemoglobin level is less than 12.0 g/dL for women and <13.0 g/dL for men after adjusting for altitude. In addition, according to the definition of the WHO, anemia can be classified as microcytic, normocytic, or macrocytic if the mean cell volume (MCV) is less than 80 fL, between 80 and 100 fL, or greater than 100 fL, respectively.[20] On the other hand, leucopenia and thrombocytopenia were defined as a WBC count less than 3×10^3 cells/μL and a platelet count less than 90×10^3 cells/μL, respectively.[21] Hypothyroidism was defined as a blood hormone level T4 or T3 less than 6.09 μg/dL, T3 less than 0.87 g/dL, or a TSH hormone level greater than 5.6 μIU/L.[22]

Ethical Consideration

After review, ethical approval was obtained from the Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar (reference number SBMLS/194/15). We conducted the current study in accordance with the Helsinki Declaration's principles. Before the data collected, all 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 or legal guardian and oral assent was obtained from children under 18 years of age after explaining the risks and benefits of the study. All other ethical issues were assured during the data collection period and after the data were collected.

Results

Sociodemographic, Behavioral and Clinical Characteristics of the Study Participants

The current study included a total of 300 participants. Of the participants, 267 (89%) were female. Approximately half of the study participants (148/49.33%) were aged 46–75 years. The mean age of the participants was 46 ± 11.96 years. More than half of the participants were from rural areas (190/63.33%) and were unable to read or write (158/52.66%). Of the participants included in this study, 41 (13.67%) were iodine salt consumers. Stool and blood film examination revealed that 11 (3.67%) participants had intestinal parasite infections, and 4 (1.33%) of the patients had malaria (Table 1).

Hematological Profiles of Patients with Hypothyroidism

After adjusting for altitude, the median Hb concentration was 14 g/dL (interquartile range (IQR): 12, 16), with a range of 4.3–18.0 g/dL. In addition, the median WBC and platelet counts were 5.3 $x10^3$/μL (IQR: 4.3, 6.1) and 228 $x10^3$/μL (IQR: 224, 233), respectively. The WBC and platelet counts ranged from 1.9 to 12.2×10^3 cells/μL and from 20–601×10^3 cells/μL, respectively. Furthermore, the median (IQR) RBC indices, such as the MCV, MCH, and MCHC, were 87 fl (84.3, 89.2), 30.7 pg (28, 33.4), and 35 g/dL (33.8, 37.6), respectively (Table 2).

Table 1 Sociodemographic, Behavioral and Clinical Characteristics of Patients with Hypothyroidism at the University of Gondar Comprehensive Specialized Hospital, Ethiopia, from January to June 2023 (n=300)

Variable	Category	Frequency (n)	Percentage (%)
Sociodemographic characteristics			
Age/years	17–30	36	12
	31–45	116	38.67
	> 45	148	49.33
Sex	Male	33	11
	Female	267	89
Marital status	Married	150	50
	Unmarried	59	19.67
	Divorced	64	21.33
	Widowed	27	8
Educational status	Unable to read and write	157	52.33
	Primary school	74	24.67
	Secondary school	60	20
	Certificate and above	9	3
Residence	Rural	190	63.33
	Urban	110	36.67
Occupational status	Housewife	166	55.33
	Civil servants	33	11
	Farmer	46	15.33
	Private worker	55	18.34
Clinical characteristics			
Iodine salt consumption	Yes	41	13.67
	No	259	86.33
History of alcohol consumption	Yes	44	14.67
	No	256	85.33
Current use of alcohol (44)	Yes	35	79.5
	No	9	20.5
Vegetable consumption	Yes	126	42
	No	174	58
Meat consumption	Yes	103	34.33
	No	197	65.37

(*Continued*)

Table 1 (Continued).

Variable	Category	Frequency (n)	Percentage (%)
History of tobacco use	Yes	11	3.67
	No	289	93.33
Current use of tobacco (11)	Yes	9	90
	No	2	10
Clinical characteristics			
Febrile illness	Yes	24	29.6
	No	57	70.4
Intestinal parasite	Yes	11	3.67
	No	289	96.33
Malaria parasite	Yes	4	1.33
	No	296	98.67

Table 2 Hematological Profile of Patients with Hypothyroidism at the University of Gondar Comprehensive Specialized Hospital, Ethiopia, from January to June 2023 (n=300)

Parameters	Median (IQR)	Range
Hb (g/dL)	14 (12, 16)	4.3–18.0
RBC (*10^6/μL)	4.63 (2.8, 6.8)	2.1–6.1
MCH (pg)	30.7 (28, 33.4)	20–38
MCHC (g/dL)	35 (33.8, 37.6)	29–40
Hct (%)	40 (37, 43.1)	16–59
MCV (fL)	87 (84.3, 89.2)	62–130
RDW (%)	14(13.4,14.6)	12–19.1
WBC (*10^3/μL)	5.3 (4.3, 6.1)	1.9–12.2
Neutrophil (*10^3/μL)	8.5(8.2,8.7)	0.8 −7.1
Lymphocyte (*10^3/μL)	2 (1.6, 2.4)	0.3–3.9
Monocyte (*10^3/μL)	0.6(0.5,0.7)	0.1–1.3
Eosinophil (*10^3/μL)	0.2(0.1,0.4)	0.1–0.9
Basophil (*10^3/μL)	0.1(0,0.1)	0–0.2
Platelet (*10^3/μL)	228 (224, 233)	20–601
MPV(fL)	8.5 (8.2, 8.7)	6.6–12.5

Abbreviations: fL, femtoliter; g/dL, gram per deciliter; Hb, hemoglobin; Hct, hematocrit; IQR, interquartile range; MCH, mean corpuscular; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; μL, microliter; pg, pictogram; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell.

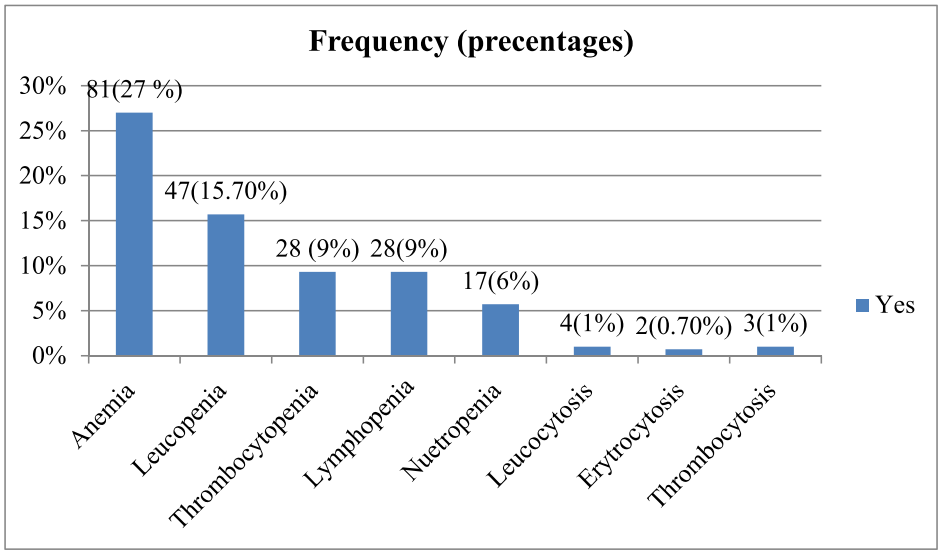

Figure 1 Magnitude of hematological abnormalities among patients with hypothyroidism at the University of Gondar Comprehensive Specialized Hospital, Ethiopia, from January to June 2023 (n=300).

The Magnitude of Hematological Abnormalities in Patients with Hypothyroidism

The overall magnitude of anemia among patients with hypothyroidism was 81 (26.3%, 95% CI: 21–32%). Among the total anemia cases, 52 (64.2%, 95% CI: 62–66%), 25 (30.86%, 95% CI: 28.5–34.5%), and 4 (4.9%, 95% CI: 3.8–5.6%) were normocytic normochromic, microcytic hypochromic, and macrocytic hypochromic, respectively. In addition, the overall incidences of leucopenia and thrombocytopenia were 47 (15.7%, 95% CI (14.2–17.2)) and 28 (9%, 95% CI (7.5–10.5)), respectively. The majority of WBC abnormalities observed in the present study were lymphopenia (28; 9%, 95% CI (8.6–10.1)) and neutropenia (17; 6%, 95% CI (4.4–7.6)) (Figure 1). On the other hand, among patients with hypothyroidism the current founds erythrocytosis, leucocytosis and thrombocytosis with the frequency of 3(1%), 4(1.3%), 3(1%), respectively (Table 3).

Table 3 Magnitude of Hematological Change in Patients with Hypothyroidism at the University of Gondar, Comprehensive Specialized Hospital, 2023 (N =300)

Hematological Parameters	Categories	Frequency (Percentage)
RBC (*10^6 /μL)	Low	20(20.5)
	Normal	237(79)
	High	3(1)
Hb (g/dL)	Low	81(26.3)
	Normal	221(73.7)
	High	2(0.7)
MCV(fl)	Low	25(30.8)
	Normal	52(64.2)
	High	4(5)

(*Continued*)

Table 3 (Continued).

Hematological Parameters	Categories	Frequency (Percentage)
RDW (%)	Low	60(20%)
	Normal	237(79)
	High	3(1)
WBC (*10^3 / L)	Low	47(15.7)
	Normal	249(83)
	High	4(1.3)
Neu count (*10^3 /μL)	Low	17(5.8)
	Normal	281(93.6)
	High	2(0.7)
Lym count (*10^3 /μL)	Low	28(9.3)
	Normal	270(90)
	High	2(0.7)
Mix count (*10^3 / μL)	Low	4(1.3)
	Normal	187(85.4)
	High	0(0)
PLT (*10^3 /μL)	Low	28(9.3)
	Normal	269(89.7)
	High	3(1)
MPV (fL)	Low	14(4.7)
	Normal	281(93.7)
	High	5(1.6)

Abbreviations: CI, confidence interval; fL, femtoliter; hematocrit; Hb, hemoglobin; g/dL, gram per deciliter; Lym, lymphocyte; MCV, mean corpuscular volume; MPV, mean platelet volume; μL, microliter; Neu, neutrophil; pg, pictogram; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; and WBC, white blood cell.

Factors Associated with Anemia

The association between anemia and hypothyroidism was assessed using both bivariate and multivariate logistic regressions. Being female increased the risk of anemia approximately twofold (AOR=2.1, 95% CI=1.1 −3.1) compared with being male. Similarly, anemia was approximately four (AOR= 3.8, CI= 1.7–8.9) times more likely to occur in patients who used alcohol than in nonusers. Patients who had febrile illness approximately three times (AOR =2.7295% CI: 1.3–5.4) more likely to develop anemia than did those without febrile disease (Table 4).

Discussion

Changes in hematological parameters such as RBC, WBC, and platelet counts are commonly observed in patients with hypothyroidism. As a result, it is important to assess the magnitude of hematological abnormalities and associated factors among patients with hypothyroidism to minimize associated complications.[23–25] The current study also aimed to assess hematological abnormalities and associated factors among patients with hypothyroidism.

Table 4 Bivariable and Multivariable Logistic Analyses of Variables Associated with Anemia Among Patients with Hypothyroidism at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, from January to June 2023

Variables	Category	Anemia Status		COR(95%, CI)	P value	AOR (95% CI)	P value
		Yes	No				
Age(years)	17–30 31–45 > 45	9 24 48	27 92 100	1[a] 0.8 (0.3–2) 1.3 (0.6–3)	0.17 0.08	1[a] 1.1 (0.9–1.3) 1.4 (1.2–1.6)	0.21 0.09
Sex	Female Male	73 8	194 25	1.18 (1.1–1.8) 1[a]	0.07	2.1(1.7–3.1) 1[a]	0.04
Marital status	Unmarried Divorced Widowed Married	17 22 6 36	42 42 21 114	1.4 (0.7–2.7) 1.2 (0.9–2.4) 1 (0.4–2.6) 1[a]	0.12 0.23 0.21	1.1 (0.6–2.5) 1.0 (0.8–2.1) 0.9(0.3–1.8) 1[a]	0.33 0.34 0.44
Residence	Rural Urban	53 28	137 82	1.13(1.1–1.8) 1[a]	0.12	1.13(1.1–1.8) 1[a]	0.14
Alcohol use	Yes No	25 56	19 200	4.6(2.5–9.1) 1[a]	0.02	3.8 (1.7–8.9) 1[a]	0.001
Vegetable use	Yes No	25 56	110 109	1[a] 2.3(1.9–2.9)	0.36	1[a] 2.1 (1.5 −4.3)	0.024
Febrile illness	Yes No	32 49	25 194	5.0 (2.8–9.6) 1[a]	0.04	2.7 (1.2–4.4) 1[a]	0.009

Note: 1[a]Reference group.

The most common hematological abnormality observed in this study was anemia (26.3%; 95% CI: 21–32%). Based on the WHO classification, the magnitude of anemia among patients with hypothyroidism in the study area was considered a moderate public health problem.[26] The magnitude of anemia was greater than that in the general population.[27] The high magnitude of anemia in patients with hypothyroidism could be related to the effect of thyroid hormones during erythropoiesis; hence, thyroid hormone regulates the production of RBCs, and the production of those hormones results in anemia.[28] In addition, the greater magnitude of anemia might be due to the age of the patients included in this study; hence, the majority (49.3%) of patients were elderly. As age advances, the function of the bone marrow decreases, which can reduce the production of RBCs and may cause anemia.

The magnitude of anemia in the current study was in line with studies performed in Kenya (28.4%)[9] and Iraq (31.3%).[20] However, these figures were greater than those of studies conducted in New York (5.9%),[29] Switzerland (5.9 and 6.54%),[30,31] Italy (7.5 and 12%),[32,33] Spain (18.6%),[34] and the UK (4.2%).[35] The reasons for these discrepancies may be related to socioeconomic status and the early treatment of anemia in those countries since most of those countries have developed standard nutrition, and early treatment of anemia in these regions may minimize the magnitude of anemia. On the other hand, the frequency was lower than that reported in studies performed in Saudi Arabia (36%, 37% and 60.27%),[15,36,37] Turkey (41%),[38] Tunisia (53.5%)[39] and India (5 and 79.5%).[40,41] The possible explanation for the variability could be attributed to variations in clinical characteristics, dietary habits, or factors that determine nutrition and health-being.[42–45]

With regard to the morphological classification of anemia, the current study revealed all types of anemia. Of these, the majority was normocytic normochromic (52 [64.2%, 95% CI: 62–66%]). These findings are supported by studies conducted in Poland, Turkey, and India.[16,38,46] This type of anemia is expected in patients with hypothyroidism. A low concentration of thyroid hormone in patients with disease is linked to the suppression of blood cell production in the bone marrow because this hormone has an effect on erythropoietin.[47,48]

In the present study, it was discovered that being female, drinking alcohol and having a febrile illness are significant aggravating factors of anemia. The association between female sex and anemia may be related to biological variability, the prevalence of thyroid disease, and menstrual loss of blood in females compared with males. On the other hand, the association between alcohol consumption and anemia might be due to the effect of alcohol on iron absorption. Alcohol inhibits the function of enzymes, which induces the production of hemoglobin. This may depress the production of RBCs directly in the bone marrow.[49–52] In addition, the association between febrile illness and anemia may be due to the immune activation of different cytokines and cells during infection, which particularly stimulate hepcidin production and capture circulating iron, respectively.[53–55]

The second most common abnormality found in this study was leucopenia (15.7%, 95% CI: 14.2–17.2), with a predominance of lymphopenia (9%, 95% CI: 8.6–10.1). Leucopenia in hypothyroidism is caused by the suppression of WBC production and differentiation because thyroid hormone is involved in this process. In addition, leucopenia may result from the destruction of WBCs by thyroid-stimulating antibodies.[56] Furthermore, thyroid hormone deficiency induces hyperproduction of reactive oxygen species (ROS) and impairs the integrity of cell surface markers. This may enhance the apoptosis of WBCs and increase the frequency of leucopenia in patients with hypothyroidism.[57,58] This figure was higher than that in a study in Kenya (12.2%).[9] These variations might be due to the variability of behavioral characteristics, clinical characteristics, and inflammatory disease.

Thrombocytopenia was identified in 2.6% (95% CI: 1.3–5.1) of the study participants. These findings are in agreement with previous studies conducted in Kenya (4.7%),[9] Japan (4.6%),[59] Iraq (4%),[20] and Poland (5%).[60] The cause of the low PLT may be the distraction of platelets by thyroid stimulation antibodies[61] and rapid consumption of platelets by hyperreactivity during thyroid dysfunction.[62]

The current study found erythrocytosis 3(1%), leucocytosis 4(1.3%), and thrombocytosis 3(1%) among patients with hypothyroidism. The finding supported by study conducted in Iraq.[20] The possible explanation for occurrence of those abnormalities may be related with increasing of chronic inflammatory conditions in some thyroid patients, these leading to the production of a various of pro-inflammatory cytokines and chemokines including IL-6, thrombopoietin, erythropoietin, and other inflammatory factors.

Limitations of the Study

As a limitation, the study examined stool samples only from saline wet mounts, which may decrease the probability of detecting intestinal parasites. The differential diagnosis of anemia was not performed. As a result of the low patient sample size, the study included a small sample size, which may affect the representativeness of the findings.

Conclusion and Recommendations

In the present study, anemia, leucopenia and thrombocytopenia were the most common hematological abnormalities. Anemia is a moderate public health factor among patients with hypothyroidism. In addition, being female, alcohol consumption, and having febrile illness were found to be significant aggravating factors of anemia. Thus, early diagnosis and follow-up strategies are needed to reduce complications, mortality and morbidity in patients with hypothyroidism.

Abbreviations

CBC, Completed Blood Count; Hb, Hemoglobin; Hct, Hematocrit; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; MCV, Mean corpuscular volume; MPV, Mean platelet volume; PDW, Platelet distribution width; RBCs, Red blood cells; RDW, Red cell distribution width; SOP, Standard Operational Procedure; T3, Triiodothyronine; T4, Thyroxine; TSH, Thyroid stimulating hormone; WBC, White blood cells; WHO, World Health Organization.

Data Sharing Statement

All the data supporting these findings are contained within the manuscript.

Consent to Participate and Ethical Approval

We confirm that all the procedures were performed in accordance with the Helsinki Declaration's principles. Ethical approval was obtained from the Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Science, the University of Gondar (SBLS/194/2015). The objective and purpose of the study were explained to the medical directors, and permission was obtained to collect the data. Written informed consent was obtained from each adult study participant to collect the data. Besides, written informed consent was obtained from the child's parent or legal guardian and oral assent was obtained from children under 18 years of age after explaining the risks and benefits of the study. No unauthorized person had access to the collected data. The findings of this study are linked to the responsible bodies.

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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.

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Disclosure

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REVIEW

The Role of Direct Oral Anticoagulants in the Treatment of Cancer-Associated Venous Thromboembolism: Review by Middle East and North African Experts

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Abstract: Venous thromboembolism is a leading cause of morbidity and mortality in patients with active cancer who require anticoagulation treatment. Choice of anticoagulant is based on careful balancing of the risks and benefits of available classes of treatment: vitamin K antagonists, low-molecular-weight heparin (LMWH), and direct oral anticoagulants (DOACs). Results from randomized controlled trials have shown the consistent efficacy of DOACs versus LMWH in the treatment of cancer-associated venous thromboembolism (VTE). However, increased major gastrointestinal bleeding was observed for edoxaban and rivaroxaban, but not apixaban, compared with LMWH dalteparin. Most guidelines recommend DOACs for the treatment of cancer-associated VTE in patients without gastrointestinal or genitourinary cancer, and with considerations for renal impairment and drug–drug interactions. These updates represent a major paradigm shift for clinicians in the Middle East and North Africa. The decision to prescribe a DOAC for a patient with cancer is not always straightforward, particularly in challenging subgroups of patients with an increased risk of bleeding. In patients with gastrointestinal malignancies who are at high risk of major gastrointestinal bleeds, apixaban may be the preferred DOAC; however, caution should be exercised if patients have upper or unresected lower gastrointestinal tumors. In patients with gastrointestinal malignancies and upper or unresected lower gastrointestinal tumors, LMWH may be preferred. Vitamin K antagonists should be used only when DOACs and LMWH are unavailable or unsuitable. In this review, we discuss the overall evidence for DOACs in the treatment of cancer-associated VTE and provide treatment suggestions for challenging subgroups of patients with cancer associated VTE.

Plain Language Summary: Patients with cancer are at risk of blood clots forming in their veins, which can cause illness and death. To prevent such blood clots, most patients with cancer need anticoagulant therapy. There are three types of anticoagulants available for the treatment of cancer-associated blood clots in a vein, namely, vitamin K antagonists, low-molecular-weight heparin (LMWH), and direct oral anticoagulants (DOACs). Drug trials have shown that DOACs are more effective than LMWH; however, DOACs can have a greater risk of causing major gastrointestinal bleeding. Among DOACs, edoxaban and rivaroxaban are drugs associated with higher rates of gastrointestinal bleeding. Recently updated guidelines for doctors recommend that DOACs be used as the first treatment for

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patients with cancer at risk of blood clot formation in a vein. For doctors in the Middle East and North Africa, this new approach differs from existing practices. Notably, choosing a treatment also depends on the type of cancer, because gastrointestinal cancers and cancers of the genitals and urinary system have an especially high risk of gastrointestinal bleeding. The choice also depends on the presence of kidney problems, drug–drug interactions, and access to the drugs. Apixaban may be the preferred DOAC in patients with gastrointestinal cancer, but this drug should be used with care in patients with upper or unresected lower gastrointestinal tumors. For patients with upper or unresected lower gastrointestinal tumors, treatment with LMWH may be preferred. Vitamin K antagonists should be used only when DOACs and LMWH are unavailable or unsuitable.

Keywords: anticoagulation, apixaban, cancer, vitamin K antagonist, low-molecular-weight heparin

Introduction

Venous thromboembolism (VTE), which includes deep-vein thrombosis (DVT) and pulmonary embolism (PE), is a well-recognized complication and a leading cause of morbidity and mortality in patients with cancer.[1–4] Active malignancy induces a prothrombotic state,[5] and the risk of developing VTE, which is approximately 4–9 times as high in cancer patients as in those without cancer, increases with the extent of the malignancy.[6–9] Active cancer, which is an independent risk factor for VTE,[10] was associated with a very high incidence rate of first VTE (5.8 per 100 person-years) in a large European population–based cohort study.[2] This study also showed that the most commonly occurring cancers (prostate, breast, lung, and colon cancers) were responsible for most active cancer-associated VTE.[2] In an American population-based cohort study, patients with active cancer had a high cumulative 5-year rate of VTE recurrence (43% compared with 27% in patients with idiopathic VTE and 18% in non-cancer secondary VTE).[11] Patients of African descent have been shown to be at greater risk of VTE than European populations. Consequently, incidence of initial and recurrent VTE in patients with cancer in the Middle East and North Africa (MENA) region is expected to be at least as high as that in Western populations, and may increase over time as diagnostic capabilities improve and anticancer therapies advance. This will eventually lead to patients with advanced, metastatic disease living longer with increasing risk of VTE.[12–14]

The treatment of VTE is particularly challenging in patients with cancer, who have a significantly greater risk of recurrent VTE and bleeding complications during anticoagulant therapy than non-cancer patients.[15] Anticoagulation treatment is further complicated by patient- and cancer-related factors that influence VTE and bleeding risk, including age, cancer stage, type of malignancy, and cancer treatment.[16,17] The clinical utility of anticoagulation is dependent on the net clinical benefit to individual patients in terms of VTE recurrence and bleeding. Therefore, after an assessment of patient- and cancer-related variables that may affect the efficacy and/or safety of anticoagulation, choice of anticoagulant is based on careful balancing of the risks and benefits of the three available classes of anticoagulation: vitamin K antagonists (VKAs), low-molecular-weight heparin (LMWH) and direct oral anticoagulants (DOACs).[17–21]

LMWH has historically been the standard of care for the treatment of cancer-associated VTE based on results from the CLOT study.[22] The CLOT study reported a statistically significant reduction of 52% in the risk of VTE recurrence after 6 months' treatment with the LMWH dalteparin versus VKA therapy (warfarin with LMWH bridging therapy), with no significant difference in major bleeding.[22] The results of individual randomized controlled trials (RCTs) have not consistently shown an efficacy benefit of LMWH over VKA therapy for the treatment of cancer-associated VTE.[22–25] However, meta-analyses of RCTs of VKA therapy versus LMWH had suggested superior efficacy of LMWH with similar safety.[17,26,27] Owing to this and also the difficulty of maintaining therapeutic VKA levels in patients with cancer, LMWH has remained the preferred treatment. Despite recommendations for the use of LMWH in patients with cancer-associated VTE, physicians in some MENA regions may have continued to prescribe VKAs for the treatment of cancer-associated VTE because of the higher cost of LMWH and patients' unease and poor compliance with long-term daily subcutaneous injections.[16,19,28,29]

Unlike VKAs, DOACs (direct factor Xa inhibitors: apixaban, edoxaban, rivaroxaban; direct thrombin inhibitor: dabigatran) can be orally administered in fixed doses and do not require regular laboratory monitoring of the anticoagulant effect.[30] As such, DOACs are standard of care for the treatment of VTE in the general population[31] but, until recently, there have been limited high-quality data on the use of DOACs in patients with cancer-associated

VTE. Over the past few years, on the basis of convincing results from RCTs of apixaban, edoxaban or rivaroxaban versus the LMWH dalteparin for the treatment of cancer-associated VTE,[32–36] these DOACs have been recognized as convenient and effective alternatives to LMWH in patients with cancer, although caution is advised in patients at high risk for bleeding, particularly in patients with gastrointestinal (GI) and genitourinary (GU) malignancies.[37–42] The decision to prescribe a DOAC for a patient with cancer is therefore not always a straightforward one, and clinicians from MENA countries may refer to guidelines from international societies such as the American College of Chest Physicians (ACCP) and the European Society of Cardiology (ESC) to inform such decisions. While recent guidelines include recommendations on DOACs for the treatment of cancer-associated VTE,[37–42] these recommendations vary according to the availability of RCT evidence at the time of publication, and certain areas lack clinical trial data to inform evidence-based recommendations.[43]

Here, we review the overall evidence for DOACs in the treatment of cancer-associated VTE and discuss the potential impact of recently published data on evidence-based recommendations. In addition to providing guidance for the use of DOACs for the treatment of cancer-associated VTE based on the most up-to-date evidence, treatment suggestions are made for challenging subgroups of patients with cancer-associated VTE where current clinical trial data to inform evidence-based recommendations may be lacking.

Evidence for DOACs in the Treatment of Cancer-Associated VTE

Although cancer patients only accounted for a small proportion of the study populations, early evidence supporting the use of DOACs in the treatment of cancer-associated VTE was obtained from the pooled subgroup of patients with cancer included in Phase 3 RCTs of DOACs (apixaban, dabigatran, edoxaban or rivaroxaban) versus conventional treatment (heparin followed by VKAs) in the general VTE population.[44–49] A meta-analysis of data aggregated from these studies in patients with cancer showed nonsignificant reductions in major and clinically relevant nonmajor (CRNM) bleeding rates and a trend toward reduction of recurrent VTE of approximately 40% with DOACs treatment.[50] However, some studies included in this meta-analysis had excluded patients with active cancers for whom treatment with LMWH would be considered more appropriate than with a VKA. Therefore, the study population included in the meta-analysis was unlikely to be representative of the DOAC target cancer patient population.[50] The studies' use of VKAs as the comparator rather than the LMWH standard of care for VTE in cancer patients further limited the relevance of the meta-analysis.

Results from five pivotal RCTs (Hokusai VTE Cancer, SELECT-D, CASTA-DIVA, Caravaggio and ADAM VTE) comparing the safety and efficacy of individual factor Xa inhibitor DOACs with the standard CLOT regimen of dalteparin (200 IU/kg for 1 month, followed by 150 IU/kg) for the treatment of cancer-associated VTE are summarized in Table 1.[32–36] The Hokusai VTE Cancer, SELECT-D, Caravaggio and ADAM VTE studies, which enrolled patients with predominantly advanced active cancer and a range of tumor types, showed ≥6 months' therapy with apixaban, edoxaban or rivaroxaban to be at least as effective as dalteparin in preventing recurrent VTE. The smaller CASTA-DIVA trial, which investigated 3 months' therapy with rivaroxaban, was unable to demonstrate noninferiority against dalteparin for the prevention of recurrent VTE.[36] There was a greater risk of major bleeding, mainly at GI sites, with edoxaban in the Hokusai VTE Cancer study and rivaroxaban in the SELECT-D study,[34,35,51] but not with rivaroxaban in CASTA-DIVA[35] or apixaban in the Caravaggio and ADAM VTE studies.[32,33,36] The PRIORITY trial, which compared the safety and efficacy of DOACs (apixaban or rivaroxaban) and dalteparin in patients with active advanced upper GI, hepatobiliary or pancreatic cancer, has provided further information regarding the use of DOACs in a high-risk patient population.[52] In this small RCT (n=90), DOACs increased the risk of clinically relevant bleeding and major bleeding compared with dalteparin, with no improvement in the rate of recurrent VTE.[52] Given that there are no known clinically meaningful differences between LMWH preparations,[53] use of dalteparin as the comparator LMWH in these RCTs does not detract from the relevance of their findings for MENA countries in which enoxaparin is the most commonly used LMWH. There are currently no published data from RCTs comparing dabigatran with LMWH for the treatment of cancer-associated VTE. A recent unblinded RCT (CANVAS) has compared the efficacy and safety between groups of patients treated with either DOACs (apixaban and rivaroxaban were used in 58.5% and 37.0% of patients, respectively) or LMWHs (enoxaparin and fondaparinux were used in 89.9% and 7.5% of patients, respectively). The DOACs were noninferior to LMWHs, with a 6-month VTE recurrence rate of 6.1% in the DOAC group and 8.8% in the LMWH group (difference,

Table 1 Characteristics and Efficacy and Safety Outcomes of Randomized Trials Comparing the Safety and Efficacy of DOACs (Apixaban, Edoxaban or Rivaroxaban) and Dalteparin in the Treatment of CAT

	Caravaggio		ADAM VTE		Hokusai VTE Cancer		SELECT-D		CASTA-DIVA	
	A (n=576)	D (n=579)	A (n=150)[a]	D (n=150)[b]	E (n=522)	D (n=524)	R (n=203)	D (n=203)	R (n=74)	D (n=84)
Age, mean (SD) or median (range) years	67.2 (11.3)	67.2 (10.9)	64.4 (11.3)	64.0 (10.8)	64.3 (11.0)	63.7 (11.7)	67 (22–87)	67 (34–87)	68.6 (62.9–77.8)[c]	70.7 (62.7–78.7)[c]
Male, n (%)	292 (50.7)	276 (47.7)	72 (48.0)	73 (48.7)	277 (53.1)	263 (50.2)	116 (57.1)	98 (48.3)	37 (50.0)	40 (47.6)
Active cancer, n (%)	559 (97.0)[d]	565 (97.6)[d]	150 (100)[e]	150 (100)[e]	513 (98.3)[f]	511 (97.5)[f]	203 (100.0)[f]	203 (100.0)[f]	74 (100.0)	84 (100.0)
Metastatic disease, n (%)	389 (67.5)[g]	396 (68.4)[g]	96 (64.0)	97 (64.7)	274 (52.5)	280 (53.4)	118 (58.1)	118 (58.1)	53/69 (76.8)	62/82 (75.6)
GI cancers, n (%)	188 (32.6)	187 (32.3)	48 (32.0)	57 (38.0)	165 (31.6)	140 (26.7)	91 (45.0)	86 (42.4)	21 (28.4)	25 (29.8)
Colorectal	121 (21.0)	113 (19.5)	18 (12.2)	29 (19.6)	83 (15.9)	79 (15.1)	55 (27.0)	47 (23.0)	13 (17.6)	19 (22.6)
Upper GI	23 (4.0)	31 (5.4)	7 (4.8)	4 (2.7)	33 (6.3)	21 (4.0)	15 (7.0)	26 (12.0)	2 (2.7)	1 (1.2)
Incidental DVT or PE, n (%)	116 (20.1)	114 (19.7)	NR	NR	167 (32.0)	173 (33.0)	108 (53.2)	105 (51.7)	36 (48.6)	32 (38.1)
Recurrent VTE, n (%)	32 (5.6)	46 (7.9)	1 (0.7)	9 (6.3)	41 (7.9)	59 (11.3)	8 (4.0)[h]	18 (11.0)[h]	4 (6.4)	6 (10.1)
HR (95% CI); DOAC vs dalteparin	0.63 (0.37–1.07)		0.099 (0.013–0.78)		0.71 (0.48–1.06)		0.43 (0.19–0.99)		0.75 (0.21–2.65)[i]	
Major bleeding, n (%)	22 (3.8)	23 (4.0)	0	2 (1.4)	36 (6.9)	21 (4.0)	11 (6.0)[h]	6 (4.0)[h]	1 (1.4)	3 (3.7)
HR (95% CI); DOAC vs dalteparin	0.82 (0.40–1.69)		0.0 (0.0–)		1.77 (1.03–3.04)		1.83 (3–11)		0.36 (0.04–3.43)[i]	
Major GI bleeding, n (%)	11 (1.9)	10 (1.7)	0	0	20 (3.8)	6 (1.1)	8 (3.9)	4 (2.0)	NR	NR
HR (95% CI); DOAC vs dalteparin	1.05 (0.44–2.50)		NE		NR		NR		NR	
CRNM bleeding, n (%)	52 (9.0)	35 (6.0)	9 (6.2)	7 (4.2)	76 (14.6)	58 (11.1)	25 (13.0)[h]	7 (4.0)[h]	8 (10.8)	5 (6.0)
HR (95% CI); DOAC vs dalteparin	1.42 (0.88–2.30)		NR		1.38 (0.98–1.94)		3.76 (1.63–8.69)		NR	
Mortality, n (%)	135 (23.4)	153 (26.4)	23 (16.0)	15 (11.0)	206 (39.5)	192 (36.6)	48 (23.6)	56 (27.6)	19 (25.7)	20 (23.8)
HR (95% CI); DOAC vs dalteparin	0.82 (0.62–1.09)		1.40 (0.82–2.43)		1.12 (0.92–1.37)		NR		1.05 (0.56–1.97)[h]	

Notes: This table collates data from the cited published papers,[32–36] also over viewed in two previously published studies[55,56] and new data available since then. [a]Primary analysis population (n=145). [b]Primary analysis population (n=142). [c]Interquartile range. [d]Cancer diagnosed within the past 6 months; receiving anticancer treatment at the time of enrollment or within the past 6 months; or recurrent locally advanced or metastatic cancer. [e]Any evidence of cancer on cross-section or PET imaging; metastatic disease; and/or cancer-related surgery, chemotherapy, or radiation therapy within the past 6 months. [f]Cancer diagnosed within the past 6 months; recurrent, regionally advanced or metastatic cancer; cancer for which treatment had been administered within the past 6 months; hematologic cancer not in complete remission. [g]Recurrent locally advanced or metastatic disease. [h]Cumulative percentages. [i]Subdistribution hazard ratio.

Abbreviations: A, apixaban; CAT, cancer-associated thrombosis; CI, confidence interval; CRNM, clinically relevant nonmajor; D, dalteparin; DOAC, direct oral anticoagulant; DVT, deep-vein thrombosis; E, edoxaban; GI, gastrointestinal; HR, hazard ratio; NE, not evaluated; NR, not reported; PE, pulmonary embolism; PET, positron emission tomography; R, rivaroxaban; SD, standard deviation; VTE, venous thromboembolism.

−2.7%; 1-sided 95% CI, −100% to 0.7%). However, noninferiority was not observed in terms of major bleeding (major bleeding events were reported in 5.2% and 5.6% of patients from DOAC and LMWH groups, respectively). A lower proportion of patients experienced severe adverse events in the DOAC group (33.8%) than in the LMWH (35.1%) group. However, death occurred in 21.5% and 18.4% of patients in the DOAC and LMWH groups, respectively. Because enoxaparin is the most commonly used LMWH in MENA countries, results from the CANVAS trial will provide important information for the clinicians in MENA.[54]

Edoxaban

The 12-month Hokusai VTE Cancer study, a noninferiority trial comparing edoxaban (n=522) with dalteparin (n=524), was the first RCT of a DOAC for the treatment of cancer-associated VTE.[34] Patients included in the trial had active cancer or a diagnosis of cancer within the previous 2 years and presented with acute symptomatic or incidentally detected proximal leg DVT or PE. Once-daily dosing of edoxaban 60 mg was initiated after ≥5 days of lead-in therapeutic-dose LMWH. Patients with body weight <60 kg, strong P-glycoprotein (P-gp) inhibitors or creatinine clearance (CrCl) 30–50 mL/min received reduced-dose edoxaban (30 mg once daily). Patients were treated for 6–12 months. The primary outcome was a composite of recurrent VTE or major bleeding during the 12 months after randomization.

The primary outcome occurred in 12.8% of patients in the edoxaban arm and 13.5% of patients in the dalteparin arm (hazard ratio [HR], 0.97; 95% confidence interval [CI], 0.70–1.36; P=0.006 for noninferiority). The primary outcome components recurrent VTE, major bleeding and CRNM bleeding were further analysed separately, and a trend toward a reduction in recurrent VTE with edoxaban versus dalteparin (HR, 0.71; 95% CI, 0.48–1.06) was observed. However, a trend toward more CRNM bleeding with edoxaban was observed, with an almost 80% increase in the risk of major bleeding versus dalteparin (HR, 1.77; 95% CI, 1.03–3.04; P=0.04). The higher rate of major bleeding with edoxaban was mostly accounted for by GI bleeding (3.8% versus 1.1%), particularly upper GI bleeding (3.3% versus 0.6%), which occurred predominantly in patients with upper or lower GI cancer. There was also a suggestion of slightly more major GU bleeding with edoxaban (0.4% versus 0% major bleeds with dalteparin). A post hoc subgroup analysis found a significant increase in major bleeding with edoxaban in patients with GI cancers (12.7% versus 3.6% with dalteparin; HR, 4.0; 95% CI, 1.5–1.06; P=0.005), whereas there was no clear increase in major bleeding with edoxaban in patients with GU cancers (4.6% versus 1.4% with dalteparin).[57] In patients with GI cancer, most (76.2%) of the 21 major bleeding events were upper GI bleeding in the edoxaban group, while none of the five major bleeding events were upper GI bleeding in the dalteparin group. Moreover, in patients with GI cancer, three-quarters of the upper GI bleeding events occurred in patients with unresected tumors, suggesting that patients with intact GI tumors were at highest risk of bleeding with edoxaban.

Rivaroxaban

Published soon after the Hokusai VTE Cancer trial results,[34] SELECT-D was a pilot study conducted to assess VTE recurrence rates in patients with active cancer treated with rivaroxaban or dalteparin.[35] A total of 406 patients presenting with acute symptomatic leg DVT or symptomatic or incidental PE were randomized to standard-dose rivaroxaban (15 mg twice daily for 3 weeks then 20 mg once daily) or dalteparin for 6 months. Compared with dalteparin, the cumulative rate of VTE recurrence at 6 months was reduced with rivaroxaban (HR, 0.43; 95% CI, 0.19–0.99), but at the cost of more bleeding, including a trend towards a doubling of major bleeding events (HR, 1.83; 95% CI, 0.68–4.96) and a marked increase in CRNM bleeding (HR, 3.76; 95% CI, 1.63–8.69). Most major bleeding events were GI bleeds, and most CRNM bleeding events were GI or GU bleeds. Patients with esophageal or gastroesophageal cancer tended to have more major bleeding events with rivaroxaban (36%) than with dalteparin (11%). Study recruitment of patients with esophageal or gastroesophageal junction cancer was stopped when the increased risk of major bleeding with rivaroxaban in patients with upper GI tumors became evident.

CASTA-DIVA was the most recently published randomized trial to compare rivaroxaban with dalteparin for the treatment of cancer-associated VTE.[36] Recruitment for this trial was stopped prematurely after randomization of 158 patients because of slow patient enrollment, which limited its statistical power. CASTA-DIVA included patients with active cancer and symptomatic or incidental proximal lower-limb DVT, iliac vein thrombosis, inferior vena cava

thrombosis and/or PE who were at high risk of recurrent VTE despite anticoagulation as estimated by a modified Ottawa score of ≥1. Patients received rivaroxaban (15 mg twice daily for 3 weeks then 20 mg once daily) or dalteparin for 3 months. The primary endpoint was the cumulative rate of VTE recurrence or worsening of pulmonary vascular or venous obstruction at 3 months, and occurred in 6.4% and 10.1% of rivaroxaban- and dalteparin-treated patients, respectively. These rates did not meet the predefined criteria for noninferiority of rivaroxaban versus dalteparin (subdistribution HR, 0.75; 95% CI, 0.21–2.66; P=0.13). Compared with dalteparin, the rate of major bleeding was numerically lower with rivaroxaban (subdistribution HR, 0.36; 95% CI, 0.04–3.43), while the rate of major bleeding or CRNM bleeding was numerically higher (HR, 1.27; 95% CI, 0.49–3.26).

In the PRIORITY trial, patients with advanced active upper GI, hepatobiliary or pancreatic cancer were randomized to a DOAC (rivaroxaban 15 mg twice daily for 3 weeks, then 20 mg once daily; or apixaban 10 mg twice daily for 7 days, and then 5 mg twice daily) or dalteparin for 6 months.[52] A total of 44 patients were assigned to the DOAC group (of whom 31 received rivaroxaban) and 46 were assigned to the dalteparin group. In a subgroup analysis by DOAC type, rivaroxaban increased the risk of clinically relevant bleeding compared with dalteparin (29.0% versus 13.0%, respectively [HR, 2.37; 95% CI, 0.84–6.66]). The risk of major bleeding was also increased (16.1% versus 4.3%, respectively [HR, 3.89; 95% CI 0.76–20.08]). The rate of recurrent VTE was 3.2% with rivaroxaban versus 2.2% with dalteparin (HR, 1.56; 95% CI, 0.10–24.93).

Apixaban

The most recently published results from RCTs comparing individual DOACs with dalteparin for the treatment of cancer-associated VTE were from the ADAM VTE and Caravaggio trials with apixaban.[32,33] The first of these studies was ADAM VTE, a small safety study that enrolled 300 patients with active cancer and VTE, including lower or upper extremity DVT or PE.[33] The second study was the Caravaggio trial, a noninferiority study in patients with symptomatic or incidental proximal lower-limb DVT or PE (n=1115).[32] In both ADAM VTE and Caravaggio, patients received standard-dose apixaban (10 mg twice daily for 7 days, followed by 5 mg twice daily) or dalteparin over 6 months.

In the ADAM VTE trial, the primary outcome was major bleeding up to 6 months, which was experienced by none of the patients receiving apixaban and 1.4% of patients receiving dalteparin (P=0.14).[33] The rate of recurrent VTE was significantly lower in patients receiving apixaban than in patients receiving dalteparin (HR, 0.099; 95% CI, 0.013–0.78; P=0.028). For the secondary composite bleeding endpoint of major or CRNM bleeding, 6% of patients in each treatment arm experienced an event.

The Caravaggio trial found apixaban to be noninferior to dalteparin with respect to the primary outcome of recurrent VTE, which occurred in 5.6% of patients treated with apixaban and 7.9% of patients in the dalteparin group (HR, 0.63; 95% CI, 0.37–1.07; P<0.001 for noninferiority, P=0.09 for superiority), with no increase in major bleeding (HR, 0.82; 95% Cl, 0.40–1.69) (Table 1).[32] Event-free survival analysis showed a significant reduction in recurrent VTE, major bleeding or death with apixaban versus dalteparin (HR, 0.74; 95% CI, 0.57–0.95), thereby reflecting a net clinical benefit. Approximately one-third of patients in Caravaggio had GI cancer, but there was no significant difference in the risk of major GI bleeding with apixaban versus dalteparin (HR, 1.05; 95% CI, 0.44–2.50). Upper and lower GI bleeding occurred in similarly low numbers of patients treated with apixaban (0.9% and 1.0%, respectively) or dalteparin (1.0% and 0.7%, respectively). A subgroup analysis of the Caravaggio trial has shown that in patients with GI cancer, rates of major GI bleeding were low and similar between the two treatment groups. In the apixaban group, lower GI bleeding occurred in 3 patients and upper GI bleeding occurred in 4 patients of the 188 patients with GI cancer, whereas in the dalteparin group, lower and upper GI bleeding occurred in 3 patients each of the 187 patients.[58] A slightly increased rate of major GU bleeding was observed with apixaban (0.7% vs 0.2%). This increase in GU bleeding contributed to a trend towards more CRNM bleeding (HR, 1.42; 95% Cl, 0.88–2.30) with apixaban versus dalteparin.[32]

Compared with the Hokusai VTE Cancer and SELECT-D trials, the small sample size, inclusion of patients with upper extremity DVT, and a slightly different distribution of cancer types in ADAM VTE, including a smaller proportion of patients with upper GI malignancy, may have resulted in a population with a relatively low risk of bleeding.[19,20,59] In contrast, patients in the Caravaggio study were an older, at-risk population with a broad range of tumor types, including upper and lower GI tumors, and a high rate of metastatic disease.[32] Rates of major bleeding in DOAC-treated patients

differed across the ADAM VTE, Caravaggio, Hokusai Cancer VTE, SELECT-D and CASTA-DIVA trials, but with the exception of ADAM VTE, major bleeding consistently occurred in 4% of patients in the dalteparin treatment arms of these trials,[32–36] indicating that, relative to ADAM VTE, the risk of bleeding was similarly high in the other trial populations.

In the PRIORITY trial of patients with advanced active upper GI, hepatobiliary or pancreatic cancer, 13 out of 40 patients randomized to the DOAC group received apixaban, and 46 patients received dalteparin. In a subgroup analysis by DOAC type, apixaban increased the risk of clinically relevant bleeding compared with dalteparin (46.2% versus 13.0%, respectively [HR 3.93; 95% CI 1.27–12.22]). The risk of major bleeding was also increased (23.1% versus 4.3%, respectively [HR 3.89; 95% CI 0.76–20.08]). There were no cases of recurrent VTE in the apixaban group and a rate of 2.2% in the dalteparin group (HR, 0.03; 95% CI, 0.0–).

A large retrospective analysis of US claims databases has generated real-world evidence to complement data from Caravaggio and ADAM VTE regarding trends toward reduced recurrent VTE and major bleeding with apixaban versus LMWH in patients with cancer-associated VTE.[60] Additionally, apixaban was shown to be associated with a lower risk of recurrent VTE than warfarin, without an increased risk of major bleeding. In this real-world analysis, patients receiving apixaban (n=3393), LMWH (n=6108) and the VKA warfarin (n=4585) had active cancer. The cohorts were well matched for characteristics that influence the risk of VTE and bleeding, including age, type of malignancy, metastases and chemotherapy. At the 6-month follow-up, a 39% reduction in the risk of recurrent VTE in the apixaban cohort versus LMWH (HR, 0.61; 95% CI, 0.47–0.81) and a 32% reduction versus warfarin (HR, 0.68; 95% CI, 0.52–0.90) was observed. Compared with LMWH, patients treated with apixaban had a 37% reduction in the risk of major bleeding (HR, 0.63; 95% CI, 0.47–0.86), as well as a lower risk of CRNM bleeding (HR, 0.81; 95% CI, 0.70–0.94). Patients treated with apixaban had less major bleeding than those receiving LMWH, regardless of metastases, cancer treatment, chemotherapy, GI cancer or VTE event type (DVT only or PE with or without DVT).[61] Compared with warfarin, apixaban-treated patients had a similar risk of major bleeding (HR, 0.73; 95% CI, 0.53–1.00) and CRNM bleeding (HR, 0.89; 95% CI, 0.77–1.04). When using the entire follow-up period (maximum length of follow-up: approximately 3 years), results were generally consistent with those of the 6-month follow-up.

Meta-Analyses

After publication of Caravaggio, a number of meta-analyses were performed in which the Caravaggio trial results were combined with the Hokusai VTE Cancer, SELECT-D and ADAM VTE trial results (Table 2).[20,51,62–66] Despite employing different statistical methods, these meta-analyses had similar findings, with the results favoring the studied factor Xa inhibitor DOACs in relation to efficacy (recurrent VTE), without a significant increase in major bleeding compared with

Table 2 Efficacy and Safety Outcomes of Meta-Analyses of the Hokusai VTE Cancer, SELECT-D, ADAM VTE and Caravaggio Trials of DOACs versus LMWH for the Treatment of CAT

Meta-Analysis	Number of Patients Analyzed	VTE Recurrence[a]	Major Bleeding[a]	CRNMB[a]
Mulder et al[64] [b]	2607	RR, 0.68; 95% CI, 0.39–1.17	RR, 1.36; 95% CI, 0.55–3.35	RR, 1.63; 95% CI, 0.73–3.64
Giustozzi et al[62]	2894	RR, 0.62; 95% CI, 0.43–0.91	RR, 1.31; 95% CI, 0.83–2.08	RR, 1.65; 95% CI, 1.19–2.28
Moik et al[20]	2894	RR, 0.62; 95% CI, 0.43–0.91	RR, 1.31; 95% CI, 0.83–2.08	RR, 1.65; 95% CI, 1.19–2.28
Tao et al[66]	2894	HR, 0.62; 95% CI, 0.43–0.91	HR, 1.31; 95% CI, 0.83–2.08	HR, 1.65; 95% CI, 1.19–2.28
Haykal et al[63]	2907	RR, 0.62; 95% CI, 0.44–0.87	RR, 1.33; 95% CI, 0.45–4.22	RR, 1.58; 95% CI, 1.11–2.24
Saleem et al[65]	2907	HR, 0.54; 95% CI, 0.23–1.28	HR, 1.38; 95% CI, 0.45–4.22	HR, 1.77; 95% CI, 0.49–6.40

Notes: This table collates data from the cited published papers,[62–66] also over viewed in two previously published studies.[55,56] [a]DOACs versus LMWH. [b]ADAM VTE not included.

Abbreviations: CAT, cancer-associated thrombosis; CI, confidence interval; CRNMB, clinically relevant nonmajor bleeding; DOAC, direct oral anticoagulant; HR, hazard ratio; LMWH, low-molecular-weight heparin; RR, risk ratio; VTE, venous thromboembolism.

dalteparin. Low heterogeneity and the consistency of efficacy results across the Hokusai VTE Cancer, SELECT-D, ADAM VTE and Caravaggio trials illustrate the generalizability of the improved efficacy of oral factor Xa inhibitors versus dalteparin.[20,62,63,67] In contrast, assessment of between-study heterogeneity for major bleeding showed that the ADAM VTE and Caravaggio trials, in which overall major bleeding risk was not increased with apixaban versus dalteparin,[32,33] were the main contributors to heterogeneity and may have influenced overall rates of major bleeding.[62] Indeed, after removal of the Caravaggio study results, meta-analysis of the remaining studies showed a significant increase in major bleeding risk with DOACs compared with dalteparin.[63] Another meta-analysis using only the Hokusai VTE Cancer and SELECT-D studies reported similar results. Compared with dalteparin, edoxaban and rivaroxaban were more effective at preventing recurrent VTE but had an increased risk of major bleeding.[51]

The CASTA-DIVA trial investigators conducted a meta-analysis incorporating CASTA-DIVA trial results with those of the Caravaggio, ADAM VTE, Hokusai VTE Cancer and SELECT-D trials.[35] Results were very similar to those shown in Table 2, with DOACs showing an overall reduced risk of recurrent VTE compared with dalteparin (HR, 0.63; 95% CI, 0.47–0.86), an increased risk of major bleeding (HR, 1.26; 95% CI, 0.84–1.90) and an increased risk of major bleeding or clinically relevant nonmajor bleeding (HR, 1.48; 95% CI, 0.49–3.26).

International Guideline Recommendations for the Treatment of Cancer-Associated VTE

Practice guidelines published before 2018 consistently recommended LMWH as the preferred treatment for cancer-associated VTE due to the absence of robust evidence for use of DOACs against LMWH. There were no or relatively weak recommendations for DOACs as an alternative to LMWH.[30] For example, local guidelines published in 2015 for the treatment of cancer-associated VTE in clinical practice in Saudi Arabia strongly recommended LMWH for the long-term treatment of VTE in patients with metastatic cancer.[28] Adherence to these now outdated guidelines would prevent many patients with active cancers from receiving anticoagulation treatment for VTE in line with the RCT data on DOACs. However, during the submission of this review, a consensus for the management of cancer-associated VTE in Saudi Arabia has been published and recommends treatment either with LMWHs or DOACs.[68] The consensus is evidence-based and specific to clinical experience, in line with the current health care policies and settings in Saudi Arabia. Therefore, this review in addition to the guidelines may benefit the other MENA countries.

Factor Xa inhibitor DOACs are now widely recommended by recent international guidelines in patients without active GI or GU cancer, who are not deemed to be at high risk of bleeding due to factors such as thrombocytopenia or renal insufficiency and are not receiving medication that may potentially cause a serious drug–drug interaction (DDI) (Table 3).[31,37–42,69] Otherwise, LMWH is preferred. However, different guidelines differ in their recommendation of which DOACs to use. The 2021 ACCP, National Comprehensive Cancer Network (NCCN) and American Society of Hematology (ASH) guidelines, all of which were published after the Caravaggio trial, support the use of apixaban, edoxaban or rivaroxaban for the treatment of cancer-associated VTE.[41,42] ACCP guidelines strongly recommend treatment with apixaban, edoxaban or rivaroxaban over LMWH, and specify that apixaban or LMWH may be preferred in patients with luminal GI malignancies.[31] Similarly, NCCN guidelines specify that apixaban, edoxaban or rivaroxaban are preferred over LMWH for patients without gastric or gastroesophageal lesions, while acknowledging that apixaban may be safer than edoxaban or rivaroxaban in patients with such lesions.[42] ASH guidelines suggest LMWH or DOACs (rivaroxaban or apixaban) for initial treatment, and DOACs (apixaban, edoxaban or rivaroxaban) for short- to long-term anticoagulation, with caution advised in patients with GI cancer because of the higher risk of bleeding.[41] With the exception of 2020 National Institute for Health and Care Excellence (NICE) guidelines, which include a general recommendation for DOACs in cancer patients,[69] guidelines published post-Hokusai VTE Cancer and SELECT-D and pre-Caravaggio specifically recommend edoxaban or rivaroxaban for patients with non-GI cancers at low bleeding risk; otherwise LMWH remains the preferred anticoagulant.[37–40] The American Society of Clinical Oncology (ASCO) guidelines support the use of apixaban, rivaroxaban, or LMWH in selected high-risk outpatients with cancer, and rivaroxaban and edoxaban for extended VTE treatment.[37] The updated 2023 ASCO guidelines for patients with cancer surgery added apixaban and rivaroxaban as options for extended pharmacologic thromboprophylaxis.[70] The International

Table 3 Guideline Recommendations for the Treatment of Cancer-Associated VTE

Guideline	Recommendations	
	Initial Treatment	**Treatment Duration**

ACCP[a]	• Apixaban, edoxaban or rivaroxaban (strong recommendation)
	  ○ Apixaban or LMWH may be preferred in luminal GI malignancies.
		• Extended-phase DOAC therapy (>3 months)
		  ○ Reassess periodically.

	• DOAC (apixaban or rivaroxaban) or LMWH (conditional recommendation)
	  ○ Caution with DOACs in GI cancers.
		• Treat for 3–6 months with a DOAC (apixaban, edoxaban or rivaroxaban) over LMWH or VKA (conditional recommendations).
		• Treat for >6 months rather than short term (3–6 months) in patients with active cancer (conditional recommendation).
		  ○ Suggest continuing indefinitely rather than stopping after completion of a definitive period of anticoagulation (conditional recommendation).
		  ○ Use a DOAC or LMWH (conditional recommendation).

NCCN[a]	• Apixaban (category 1), edoxaban after ≥5 days of parenteral anticoagulation (category 1) or rivaroxaban (category 2A) preferred for patients without gastric or gastroesophageal lesions ○ Caution in GU tract lesions • LMWH preferred for patients with gastric or gastroesophageal lesions (category 1). • Dabigatran if above regimens are not appropriate or unavailable.	• ≥3 months or as long as active cancer or cancer therapy.

ASCO[b]	• LMWH, UFH, fondaparinux, rivaroxaban, or apixaban
	  ○ For long-term anti-coagulation, LMWH, edoxaban, rivaroxaban, or apixaban for at least 6 months are preferred over VKAs. Caution with direct factor Xa inhibitors in patients with GI and GU cancers or other high-risk settings.
		• Offer LMWH, DOACs or VKAs beyond the initial 6 months to select patients with active cancer, such as those with metastatic disease or those receiving chemotherapy.
		  ○ LMWH, edoxaban or rivaroxaban preferred.
		  ○ LMWH preferred in settings with increased bleeding risk.
		• Assess intermittently to ensure a continued favorable risk-benefit profile.
		• Patients needing extended pharmacologic antithrombotic prophylaxis post cancer surgery
		  ○ Prophylactic doses of LMWH

ESC[b]	• PE and cancer: LMWH for the first 3–6 months (IIa. A) • Edoxaban (IIa. B) or rivaroxaban (IIa. C) may be used except in GI cancer patients.	• Extend indefinitely or until the cancer is cured (IIa. B). • Consider LMWH, DOAC or VKA.
ITAC[b]	• LMWH when CrCl ≥30 mL/min (grade 1A). • Apixaban or rivaroxaban (first 10 days) or edoxaban (started after initial LMWH/UFH for 5 days) can be used for initial treatment if CrCl ≥30 mL/min and patient is not at high risk of GI or GU bleeding (grade 1 A).	• LMWH or DOACs for ≥6 months (grade 1 A) ○ DOACs when CrCl ≥30 mL/min if no impairment in GI absorption or strong DDIs (grade 1 A), but caution advised in GI malignancies, especially upper GI tract. • After 6 months, termination or continuation of anticoagulation based on benefit–risk ratio, tolerability, drug availability, patient preference and cancer activity (guidance).
ISTH[b]	• Patients with low bleeding risk and no DDIs: edoxaban or rivaroxaban; LMWHs are acceptable alternatives. • Patients with high bleeding risk[c]: LMWH; edoxaban or rivaroxaban as an alternative if no potential DDI.	• No specific recommendation.

(*Continued*)

Table 3 (Continued).

Guideline	Recommendations	
	Initial Treatment	**Treatment Duration**
NICE[b]	• Consider DOAC if active cancer and confirmed proximal DVT or PE. • If DOAC unsuitable, consider LMWH alone or VKA (following initial LMWH). • Choice of anticoagulant should consider tumor site, DDIs and bleeding risk.	• Review treatment at 3 to 6 months according to clinical need.
ESMO	• During the acute phase (first 5–10 days after diagnosis) ○ Consider LMWH, UFH, fondaparinux, apixaban or rivaroxaban (I, A) ○ LMWH is preferable to UFH and fondaparinux (V, A)	• For long-term anticoagulation for at least 6 months ○ LMWH, apixaban, edoxaban or rivaroxaban are preferred to VKAs (I, A) ○ In patients with luminal GI cancer, urothelial cancer (II, B), patients at high risk of GI bleeding, receiving powerful inducers and/or inhibitors of CYP3A4 or P-gp, (IV, B) LMWH is preferred. • Beyond initial 6 months, LMWH, apixaban, edoxaban, rivaroxaban or VKAs based on benefit–risk assessment (III, B) • Regularly assess risk–benefit profile of anticoagulation therapy for favorable balance (IV, C)

Notes: This table collates data from the cited published papers,[31,37–42,69,72] also over viewed in two previously published studies[55,56] and new data available since then. [a]Recommendations based on ADAM VTE, Caravaggio, Hokusai VTE Cancer and SELECT-D trial results. [b]Recommendations based on Hokusai VTE Cancer and SELECT-D trial results. [c]High bleeding risk includes patients with luminal gastrointestinal cancers with an intact primary; cancers at risk of bleeding from the genitourinary tract, bladder, or nephrostomy tubes; or active GI mucosal abnormalities (eg, duodenal ulcers, gastritis, esophagitis, or colitis).

Abbreviations: ACCP, American College of Chest Physicians; ASCO, American Society of Clinical Oncology; CrCl, creatinine clearance; CYP3A4, cytochrome P450 3A4; DDI, drug–drug interaction; DOAC, direct oral anticoagulant; ESC, European Society of Cardiology; ESMO, European Society for Medical Oncology; GI, gastrointestinal; GU, genitourinary; ISTH, International Society on Thrombosis and Haemostasis; ITAC, International Initiative on Thrombosis and Cancer; LMWH, low-molecular-weight heparin; NICE, National Institute for Health and Care Excellence; NCCN, National Comprehensive Cancer Network; P-gp, P-glycoprotein; PE, pulmonary embolism; UFH, unfractionated heparin; VKA, vitamin K antagonist; VTE, venous thromboembolism.

Initiative on Thrombosis and Cancer (ITAC) guidelines, published in 2019 and updated in 2022, recommend the use of LMWHs or DOACs as initial treatment of established VTE, depending on the creatinine clearance and patient's risk of GI or GU cancer.[40,71] For early maintenance and long-term treatment, the 2022 guideline provides an update that LMWHs are preferred but apixaban, rivaroxaban and edoxaban can be used, depending on creatinine clearance and with caution in patients with GI or GU cancers.[40,71] The 2023 European Society for Medical Oncology (ESMO) clinical practice guidelines for VTE in cancer patients recommend the use of LMWH, unfractionated heparin (UFH), fondaparinux, apixaban or rivaroxaban for acute phase (first 5–10 days after diagnosis), with a preference for LMWH than UFH and fondaparinux.[72] For long term, the use of LMWH, apixaban, edoxaban or rivaroxaban is preferred over VKA. In patients with luminal GI cancer, patients at high risk of GI bleeding, receiving inducers and/or inhibitors of cytochrome P450 3A4 (CYP3A4) or P-gp, LMWH is preferred.[72] Beyond the initial 6 months, the ESMO guidelines recommend LMWH, apixaban, edoxaban, rivaroxaban or VKAs if patients have active cancer and their risk of thrombosis outweighs the risk of bleeding.[72] Going forward, the Caravaggio trial, which has clearly established the important role of DOACs, especially apixaban, in the treatment of cancer-associated VTE,[67] will contribute to the guidelines as they are updated.

Because high-quality data for extended anticoagulation treatment are limited to the 6- to 12-month time period, recommendations regarding its extended use in cancer patients are mostly based on expert opinion.[73] Although the optimal duration of DOAC or LMWH therapy is uncertain, there is general consensus that anticoagulation should be continued for ≥3–6 months or, given the high long-term risk of recurrent VTE in patients with active cancer,[11] for as long as the cancer is active or being treated, unless there is a contraindication or unacceptable clinical risk.[37–40,42] Extended anticoagulation is much more achievable with a DOAC than with LMWH therapy, which is often discontinued after 3–6 months.[74,75] The recently completed 12-month EVE trial (NCT03080883) found similar rates of bleeding with apixaban 5 mg or 2.5 mg twice daily without increasing thrombotic outcomes.[76] Additionally, an extended DOAC treatment study

API-CAT (NCT03692065) comparing standard-dose apixaban (5 mg twice daily) with stepped down 2.5 mg dosing is ongoing.[73] An ideal future study would be to compare a DOAC versus placebo after 6 months of standard treatment if the cancer is no longer active or if other thrombotic risk factors are resolved.

DOACs in Challenging Subgroups of Cancer Patients

Although recent cancer-associated VTE treatment guidelines include recommendations for the use of DOACs, many patient management questions remain unanswered due to the lack of clinical trial data.[77] Patients with certain tumor types, thrombocytopenia, renal or hepatic impairment, and any other condition or concomitant medication associated with an increased risk of bleeding or unclear net clinical benefit for anticoagulation provide clinical challenges for which empiric management decisions must be made.[17,43,78] To date, the shift towards using DOACs in these challenging subgroups of patients has been appropriately cautious, and guidance on their use is required (Table 4).[43]

Table 4 Guidance for the Use of DOACs to Treat Cancer-Associated VTE in Challenging Patient Subgroups

Patient Subgroup	Treatment Suggestions
GI cancer	• LMWH preferred in patients with intact primary lower GI tumors and patients with upper GI tumors. • Apixaban suggested for patients with resected primary lower GI tumors and patients who refuse LMWH or prefer oral therapy. • LMWH preferred after proximal GI surgery for tumor resection.
GU cancer	• LMWH preferred. • DOAC (apixaban, edoxaban or rivaroxaban) suggested for patients who refuse LMWH or prefer oral therapy.
Intracranial tumors	• Standard anticoagulation with a DOAC (apixaban, edoxaban or rivaroxaban) or LMWH.
Concomitant anticancer treatment	• Check for potential DDIs before DOACs are used in patients receiving chemotherapy or targeted cancer therapies. • Consider LMWH if DDIs are a concern with DOACs.
Concomitant antiplatelet agents	• DOAC (apixaban preferred) combined with one antiplatelet agent ($P2Y_{12}$ inhibitor) in the event of cancer-associated VTE and a new coronary stent. • Start DOAC (apixaban preferred) and stop one antiplatelet agent in patients receiving dual antiplatelet therapy for CAD.
Low body weight (≤60 kg)	• Full-dose apixaban or rivaroxaban. • Half-dose edoxaban.
Renal impairment	• CrCl ≥30 mL/minute: apixaban, edoxaban (half-dose for CrCl 30–50 mL/min) or rivaroxaban. • CrCl <30 mL/minute: dose-adjusted LMWH (with anti-Xa level monitoring) or consider apixaban (unless CrCl <25 mL/min).
Hepatic impairment	• Avoid DOACs in patients with Child-Turcotte-Pugh class B or C cirrhosis. • Apixaban and edoxaban: avoid if total bilirubin (not due to Gilbert's syndrome) >1.5 × ULN or transaminases >2 × ULN). • Dabigatran or rivaroxaban: avoid if transaminases >3 × ULN.
Thrombocytopenia	• Platelet count >50 × 10^9/L: full-dose DOAC. • Platelet count <25 × 10^9/L: hold anticoagulation. • Platelet count 25–50 × 10^9/L: dose-adjusted LMWH (preferred) or half-dose DOAC (apixaban, edoxaban or rivaroxaban).
Incidental VTE	• Treat in the same manner as symptomatic VTE (DOAC or LMWH according to bleeding risk and patient preference).

Abbreviations: CAD, coronary artery disease; CrCl, creatinine clearance; DDI, drug–drug interaction; DOAC, direct oral anticoagulant; GI, gastrointestinal; GU, genitourinary; LMWH, low-molecular-weight heparin; ULN, upper limit of normal; VTE, venous thromboembolism.

GI Cancer

In patients with GI cancer, edoxaban and rivaroxaban use is not recommended because of an increased risk of major GI bleeding versus dalteparin in the Hokusai VTE Cancer and SELECT-D trials.[34,35] However, the Caravaggio trial does not report an increased risk of major upper or lower GI bleeding with apixaban versus dalteparin. This Caravaggio trial observation suggests that apixaban could be a safe alternative to LMWH in patients with GI cancer.[32,78] It is unclear whether apixaban is safer than edoxaban or rivaroxaban in patients with GI cancer. However, it is possible that more stable plasma drug concentrations, lower peaks and higher troughs, obtained with twice-daily apixaban dosing compared with once-daily edoxaban or rivaroxaban dosing, could have an impact on safety[79,80] and contribute to reduced risk of GI bleeding. Although the mechanism of bleeding in GI cancer patients treated with DOACs remains unclear, in the Hokusai VTE Cancer study, upper GI bleeding was reported in all types of GI cancer regardless of location,[57] suggesting a possible direct effect on the upper GI tract.

Both the Hokusai VTE Cancer and Caravaggio trials had similar proportions of patients with GI cancer (~30%), and in both studies, approximately 5% of the total patients had upper GI cancer.[32,34] Most of the GI bleeding events from the Hokusai VTE Cancer trial (patients receiving edoxaban) and all major GI bleeding events from the Caravaggio trial (patients receiving apixaban) occurred in patients with unresected tumors.[58] Therefore, although results with apixaban from the Caravaggio trial are promising, apixaban should be used with caution in patients with GI cancer, particularly those with intact primary GI tumors.

Results of the PRIORITY trial, in which approximately 59% of patients had upper GI cancer, provide further evidence that caution is needed when choosing an anticoagulant for this patient population. Although the PRIORITY sample size was small, both apixaban and rivaroxaban increased the risks of clinically relevant bleeding and major bleeding compared with dalteparin. A total of 52% of clinically relevant bleeding events and 80% of major bleeding events occurred in the GI tract. In this trial, cancer involvement at the GI mucosa rather than the cancer type was a significant risk factor for clinically relevant bleeding.

Stomach cancer is the most common GI cancer in the MENA region, followed by liver cancer, colorectal cancer, and esophageal cancer, but rates of gastric and esophageal cancers vary between countries according to genetic and lifestyle factors.[81] On the basis of the available evidence, LMWH should still be the anticoagulant of choice in patients with gastric or esophageal cancer, and apixaban should be the preferred DOAC when these high-risk patients refuse parenteral therapy.[78]

There are also limited data for the use of DOACs in patients after proximal GI surgery for tumor resection. In situations such as this, when GI absorption may be compromised, LMWH is often preferred to ensure adequate levels of anticoagulation.[43,78]

GU Cancer

In general, treatment guidelines acknowledge that the risk of GU tract bleeding may increase with DOACs, and advise caution in patients with active GU tract lesions.[43] The Caravaggio and Hokusai VTE Cancer trial results[32,34] suggested increased GU bleeding with apixaban and edoxaban but numbers were too small for meaningful analysis and, therefore, a conclusion regarding GU bleeding risk could not be drawn. Meta-analyses of subgroup data collected from the Hokusai VTE Cancer, SELECT-D, ADAM VTE and Caravaggio trials also showed that GU sites of major bleeding were more common with factor Xa inhibitor DOACs than with dalteparin.[20,62] However, in patients with GU cancer, these DOACs were not significantly associated with a greater risk of major bleeding versus dalteparin (RR, 2.81; 95% CI, 0.45–17.40; P=0.27).[63]

Urothelial irritation from schistosomiasis infection contributes to high rates of squamous cell carcinoma of the bladder in some MENA countries, but a gradual shift to more urothelial carcinomas linked to cigarette smoking and other lifestyle changes linked to urbanization has been observed.[82–84] Based on current evidence of a possible increase in the risk of urothelial bleeding with DOACs, LMWH may be preferred in patients with active, unresected luminal lesions in the GU tract, recent GU tumor surgery or recent major GU bleeding.[78] When DOACs are used in such patients, vigilance for signs of urothelial bleeding is required.

Intracranial Tumors

Patients with intracranial tumors have an increased risk of VTE and also an increased risk of intracranial hemorrhage.[78] In the Caravaggio trial, patients with brain tumors or intracerebral metastases were excluded as a precaution.[32] A retrospective cohort study in patients with primary or metastatic brain tumors with brain lesions published after the start of Caravaggio

showed that DOACs did not increase the risk of intracranial hemorrhage compared with LMWH.[85] Therefore, patients who developed cerebral metastases during the Caravaggio trial were allowed to continue treatment. Furthermore, in the Hokusai VTE Cancer trial, which included 74 (7.1%) patients with primary or metastatic brain tumors, a similar low percentage of patients from the edoxaban (0.4%) and dalteparin (0.6%)[57] groups experienced intracranial hemorrhage. In a recent retrospective study, numerically lower incidence rates and higher reduction in incidence of recurrent VTE, major bleeding, and CRNM bleeding were observed for apixaban versus LMWH in patients with brain cancer.[86] Therefore, DOACs are considered to be at least as safe as LMWH in patients with primary or metastatic brain tumors.[78]

Concomitant Cancer Treatment

Although DOACs have fewer DDIs than VKAs[77] do, all DOACs are substrates of P-gp. In addition, apixaban and rivaroxaban are also substrates of CYP3A4.[87] Chemotherapies or targeted cancer therapies that affect P-gp and/or CYP3A4 pathways may therefore decrease or increase the anticoagulant effect of DOACs, and care is needed during concurrent treatment. Patients treated with powerful inducers and/or inhibitors of CYP3A4 or P-gp have been primarily excluded from RCTs of DOACs for the treatment of cancer-associated VTE;[59,78] however, no anticancer therapy was excluded from Caravaggio. As such, patients were receiving a broad array of cytotoxic and biologic anticancer therapies, including new therapies, such as antiangiogenic monoclonal antibodies (~3% of patients) and checkpoint inhibitors (~2%).[32] Concomitant administration of anticancer agents, including P-gp and/or CYP3A4 inhibitors or inducers, did not appear to affect the incidence of VTE recurrence and major bleeding associated with apixaban. This observation suggests that apixaban can be safely administered in patients with cancer-associated VTE even when they are receiving concomitant anticancer treatment.[88] Despite this, uncertainty remains regarding DDIs between DOACs and cancer therapies, and potential DDIs should be checked before DOACs are used in patients receiving chemotherapy or targeted cancer therapies.[18,59,78] In patients receiving concurrent strong dual CYP3A4 and P-gp inhibitors, a dose reduction to 2.5 mg twice daily is recommended for apixaban. Similarly, in patients on concurrent potent P-gp inhibitors, a dose reduction to 30 mg daily for edoxaban is recommended.[78] DOACs should be avoided in all other instances in which DDI is a concern.[18,78] In some practices, DOACs are avoided on the day of chemotherapy because of uncertainty about reactions between chemotherapy and DOACs; on these days, patients receive LMWH.

Concomitant Antiplatelet Agents

Antiplatelet agents are frequently indicated for coronary artery disease, which is a common comorbidity in cancer patients worldwide.[89,90] There are no cancer-specific data to guide combined anticoagulant and antiplatelet therapy in patients with cancer-associated VTE and cardiovascular disease; thus, suggestions for treatment must be extrapolated from the best available evidence in the general population.[78] An antiplatelet subgroup analysis of the AMPLIFY study showed that an improved safety profile of apixaban compared with VKA was seen in patients with VTE whether or not they were taking antiplatelet agents.[91] The AUGUSTUS trial in patients with atrial fibrillation and recent acute coronary syndrome or percutaneous coronary intervention showed that apixaban plus a P2Y12 inhibitor antiplatelet agent resulted in fewer bleeding complications and hospitalizations than combination therapy with a VKA and a P2Y12 inhibitor with or without aspirin.[92] On the basis of these findings, it has been suggested that patients with cancer associated thrombosis and a new coronary stent receive a DOAC, preferably apixaban, in combination with a PY12 inhibitor.[78] In patients with stable coronary artery disease who experience cancer-associated VTE while receiving dual antiplatelet therapy, it is suggested that one of the antiplatelet agents is stopped and a DOAC be started.[78] There are no data for LMWH in this population.

Low Body Weight

DOACs may be considered in cancer patients with low body weight (≤60 kg). Although half-dose edoxaban was used in patients with body weight ≤60 kg in the Hokusai VTE Cancer trial,[34] there is no strong evidence for DOAC dose reduction in low-weight patients. All patients from the SELECT-D, CASTA-DIVA, ADAM VTE and Caravaggio trials[32,34–36] received full-dose rivaroxaban or apixaban. On the basis of these trials, clinicians should feel comfortable using standard doses of these DOACs regardless of body weight.[43]

Obesity

In patients with cancer, obesity might increase the risk of VTE. Limited research is available on the efficacy and safety of DOACs in patients with cancer and obesity. A post-hoc analysis of data from the AVERT trial was conducted to investigate the efficacy and safety of apixaban thromboprophylaxis in obese (n=215) versus non-obese (n=348) patients with cancer. Patients were classified as obese (body mass index [BMI] ≥30 kg/m^2) and non-obese (BMI <30 kg/m^2) based on their BMI at randomization. Among the non-obese patients, 9 (5.1%) patients out of 178 receiving apixaban and 15 (8.8%) out of 170 patients receiving placebo had VTE. Among the obese patients, a significantly lower incidence of VTE was observed in patients receiving apixaban (n=4/110) than in patients receiving placebo (n=14/105) (HR, 0.26; 95% CI, 0.14–0.46; P<0.0001). Similarly, lower risks of PE and DVT were observed in patients treated with apixaban than placebo. A numerically higher but statistically non-significant risk of clinically relevant bleeding was observed in patients receiving apixaban than placebo (HR, 2.09; 95% CI, 0.96–4.51; P=0.062).[93] However, interpretation of these results requires caution because the distribution of types of cancer with distinct thrombo-hemorrhagic risk profiles was significantly different between the groups. Another study assessed outcomes in patients with morbid obesity, acute VTE, and concurrent cancer receiving anticoagulation treatment from the Registro Informatizado Enfermedad TromboEmbólica.[94] The patients were receiving anticoagulation either with VKA, LMWH, or DOACs. A lower mortality rate (HR, 0.34; 95% CI, 0.25–0.45) and lower rate of major bleeding (HR, 0.54; 95% CI, 0.28–0.96) were observed in patients with cancer and obesity (n=245) than in patients with cancer and had normal weight (n=4198), while the rate of VTE recurrence was similar between these groups (HR, 0.62; 95% CI, 0.34–1.05).[94] Specific research on the efficacy and safety of DOACs in patients with cancer and obesity is lacking. However, these limited data point towards a possible use of DOACs in patients with cancer and obesity.

Renal Impairment

Renal insufficiency may develop in patients with cancer as a consequence of malignancy. Severe renal insufficiency treatment or associated complications increase the risk of VTE and bleeding.[78,95] Most DOACs are at least partially renally cleared, and pivotal DOAC RCTs both in general and cancer populations excluded patients with CrCl <30 mL/min (<25 mL/min for apixaban).[78] In general, no dose adjustment is required in patients with mild renal insufficiency.[43] The edoxaban dose is halved in patients with CrCl 30–50 mL/min.[34] Apixaban is the only DOAC approved for use in patients with CrCl <30 mL/min, but questions remain as to the optimal use of the drug in this population. Dose-adjusted LMWH (with anti-Xa monitoring) is an appropriate alternative in patients with CrCl <30 mL/min.[17,78]

Hepatic Impairment

Guidance for the use of DOACs in patients with liver disease is important in the MENA region, which has a high burden of chronic liver disease and cirrhosis secondary to hepatitis C infection.[81,96,97] DOACs are not recommended for patients with Child-Turcotte-Pugh class B or C cirrhosis.[17] Apixaban and edoxaban are contraindicated in patients with clinically significant liver disease, including transaminases >2 × upper limit of normal (ULN), and rivaroxaban is contraindicated in patients with transaminases >3 × ULN.

Thrombocytopenia

Thrombocytopenia resulting from chemotherapy and/or malignancy is common in patients with cancer. In this setting, the risk of bleeding is increased but the risk of VTE remains.[78] In the Caravaggio trial, owing to the increased risk of bleeding associated with low platelet counts, patients with acute leukemia were excluded. Moreover, all five RCTs comparing DOACs with dalteparin in patients with cancer-associated VTE excluded patients with severe thrombocytopenia (Hokusai VTE Cancer and ADAM VTE trials: platelet count <50 × 10^9/L; SELECT-D trial: <100 × 10^9/L; CASTA-DIVA trial: <50 × 10^9/L; Caravaggio trial: <75 × 10^9/L).[32–36] For patients with platelet count <25 × 10^9/L, it is safest to withhold anticoagulation until the platelet count has recovered.[78] In the absence of data on the use of DOACs in patients with cancer-associated VTE and platelet count <50 × 10^9/L, dose-adjusted LMWH may be preferred for patients with a platelet count 25–50 × 10^9/L, but a half-dose DOAC has been suggested as a potentially acceptable alternative.[78] Care should be taken when using DOACs in patients undergoing chemotherapy and a decrease in platelet counts is expected.[17]

Incidentally Diagnosed VTE

In some MENA regions, because of the use of high-resolution CT scanning for staging and follow-up of cancer patients, the incidence of incidentally diagnosed VTE is increasing. Incidental VTE was reported as the index event in approximately 20% to 50% of patients in the Hokusai VTE Cancer, SELECT-D, CASTA-DIVA and Caravaggio trials.[32,34–36] Subgroup meta-analysis of data from the Hokusai Cancer VTE and Caravaggio trials with or without inclusion of SELECT-D showed that the risks of recurrent VTE and major bleeding with DOACs versus dalteparin were similar in patients with either incidental or symptomatic VTE as the index event, further favoring DOACs in terms of efficacy, without a significant increase in major bleeding.[20,64] Although clinicians may question whether all incidentally discovered asymptomatic VTE should be treated with full-dose DOAC therapy,[17] incidental VTE can generally be treated in the same manner as symptomatic VTE.[37,42]

Discussion

Factor Xa inhibitor DOACs are recommended for the treatment of VTE in patients with non-GI cancers at low bleeding risk with no potential for DDIs.[31,37–42,69] Current practice favors LMWH in all other patients. The more recent Caravaggio and ADAM VTE trial results have added to the evidence from Hokusai Cancer VTE and SELECT-D trials of the consistent efficacy of factor Xa inhibitor DOACs for the treatment of cancer-associated VTE but have also showed some heterogeneity with regard to safety.[32–35] Concerning major bleeding and major GI bleeding, the Caravaggio and ADAM VTE study results with apixaban compared favorably with those of the Hokusai VTE Cancer and SELECT-D trials of edoxaban and rivaroxaban, clearly demonstrating no increased risk of bleeding with apixaban versus dalteparin in contrast to increased risk with edoxaban or rivaroxaban. The efficacy and safety outcomes of rivaroxaban versus dalteparin in CASTA-DIVA were generally consistent with those of the Cancer VTE, SELECT-D, Caravaggio and ADAM VTE trials, despite the small sample size limiting statistical power in this trial.[36] However, in the absence of any RCTs comparing the DOACs head-to-head, it is inappropriate to conclude that any one DOAC is safer than another.

The Caravaggio trial is a landmark study that has clearly established the important role of DOACs in the treatment of cancer-associated VTE,[67] but with the exception of the ACCP, ASH and NCCN 2021 guideline recommendations,[31,41,42] all major international practice guidelines for the treatment of CAT currently pre-date publication of the Caravaggio trial results.[37–40,69] These guidelines tend to recommend edoxaban or rivaroxaban as preferred therapy for VTE in patients with non-GI cancer.[37–40,42] The Caravaggio study finding that apixaban was noninferior to dalteparin for the treatment of cancer-associated VTE without an increased risk of major lower or upper GI bleeding, despite approximately one-third of the population having GI cancer,[32] will contribute to the evolution of practice guidelines as they are updated. In the absence of up-to-date MENA-specific guidelines, physicians in MENA regions are advised to adhere to recommendations from the most up-to-date international guidelines[31,41,42] and the most recent Saudi Arabia guidelines.[68]

The results of the Caravaggio trial should help clinicians to make informed decisions for the treatment of cancer-associated VTE. Regardless of tumor type, clinicians should consider prescribing a factor Xa inhibitor DOAC as a practical, long-term alternative to LMWH for patients with active cancer. Treatment should be extended beyond 6 months in patients with ongoing active malignancy such as metastatic disease and/or with ongoing anticancer therapy, and it should be assessed periodically to ensure continued net clinical benefit. The Caravaggio trial and real-world evidence support a recommendation for apixaban in patients with GI tumors, but caution is advised, particularly in patients with upper GI tumors or unresected lower GI tumors.[31,32,42,58,78] Ultimately, the decision to use a DOAC requires careful consideration of bleeding risk, the cost–benefit and convenience of oral therapy, and patient needs and preferences, and these factors may change over the course of the cancer journey. This also applies when deciding whether to use a DOAC in other challenging subgroups of patients, including patients with GU cancers and patients with comorbidities that increase the risk of bleeding, such as patients with severe renal impairment or thrombocytopenia. LMWH may be preferred for patients at very high risk of bleeding who are willing and able to comply with daily subcutaneous injections. VKAs should only be used in patients for whom DOACs and LMWH are unavailable or unsuitable.

Conclusion

DOACs represent a major paradigm shift in the treatment of cancer-associated VTE in the MENA region. Cancer patients already have a major burden of illness, and long-term anticoagulation is generally much more achievable with oral anticoagulation than with LMWH. Therefore, in countries where they are available, DOACs should always be considered for the treatment of cancer-associated VTE. However, in patients with GI malignancies and upper or unresected lower GI tumors, LMWH may be preferred due to increased risk of major GI bleeding events. Vitamin K antagonists should be used only when DOACs and LMWH are unavailable or unsuitable.

Abbreviations

ACCP, American College of Chest Physicians; ASCO, American Society of Clinical Oncology; ASH, American Society of Hematology; CI, confidence interval; CrCl, creatinine clearance; CRNM, clinically relevant nonmajor; D, dalteparin; DDI, drug–drug interaction; DOAC, direct oral anticoagulant; DVT, deep-vein thrombosis; E, edoxaban; ESC, European Society of Cardiology; ESMO, European Society for Medical Oncology; GI, gastrointestinal; GU, genitourinary; HR, hazard ratio; ISTH, International Society on Thrombosis and Haemostasis; ITAC, International Initiative on Thrombosis and Cancer; LMWH, low-molecular-weight heparin; MENA, Middle East and North Africa; NCCN, National Comprehensive Cancer Network; NE, not evaluated; NICE, National Institute for Health and Care Excellence; NR, not reported; PE, pulmonary embolism; PET, positron emission tomography; P-gp, P-glycoprotein; R, rivaroxaban; RCT, randomized controlled trials; RR, risk ratio; SD, standard deviation; UFH, unfractionated heparin; ULN, upper limit of normal; VKA, vitamin K antagonist; VTE, venous thromboembolism.

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Author Contributions

Authors H.M.E-Z., T.O., M.A., A.W., M.M., S.B., F.H., and A.C. conceptualized the study. Authors A.W., M.M., E.D., H.J., S. M.S., F.H., and A.C. conducted the formal data analysis. The original draft was prepared by authors M.M., F.H., and A.C. All authors interpreted the data, revised and critically reviewed the article. All authors agreed on the journal to which the article was submitted. All authors gave approval to the final published version and agree to be accountable for all aspects of the work.

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ORIGINAL RESEARCH

Risk of Intracranial Hemorrhage in Persons with Hemophilia A in the United States: Real-World Retrospective Cohort Study Using the ATHNdataset

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Introduction: Intracranial hemorrhage (ICH), a serious complication in persons with hemophilia A (PWHA), causes high rates of mortality and morbidity. Identified ICH risk factors from patient data spanning 1998–2008 require reassessment in light of changes in the current treatment landscape.

Aim and methods: PWHA identified in the ATHNdataset were evaluated retrospectively to assess incidence of ICH and determine the association between ICH risk and key characteristics using time-to-event analyses (Cox proportional-hazards models, survival curves, and sensitivity analyses).

Results: Over a median follow-up time of 10.7 patient-years, 135 of 7837 PWHA over 2 years of age in the ATHNdataset (1.7%) experienced an ICH. Stratification by prophylaxis status and inhibitor status resulted in an incidence rate (IR) ratio (IRR) (IR+/IR−) of 0.63 (95% confidence interval [CI], 0.43–0.94; *P*=0.020) and 1.76 (95% CI, 0.97–3.20; *P*=0.059), respectively. Characteristics associated with greater risk of developing ICH include being aged 2–12 years; being covered by Medicaid; having had HIV, hepatitis C, or hypertension; and never having received factor VIII or prophylactic treatment. In multivariable analysis with interaction, the estimated hazard ratio for PWHA never receiving prophylaxis was 7.67 (95% CI, 2.24–26.30), which shrunk to 2.03 (95% CI, 1.30–9.12) in bootstrapping analysis and 3.09 in the highest-penalty ridge-regression analysis but was still significant. Inhibitor status was found not to be statistically associated with ICH in all analyses.

Conclusion: These results align with previous studies demonstrating that prophylaxis confers a protective effect against ICH. Previously, inhibitor positivity had been shown to increase risk for ICH; however, this study did not corroborate those findings.

Keywords: bleeding, factor VIII, health insurance, hematologic disease, prophylaxis, risk factors

Introduction

Hemophilia A (HA) is an X-linked recessive bleeding disorder caused by a deficiency of factor VIII (FVIII).[1,2] There are an estimated 24,000–26,400 males with hemophilia A in the United States; approximately 75% of persons with hemophilia A (PWHA) have moderate or severe forms of the disease.[3,4] Intracranial hemorrhage (ICH) in PWHA is associated with a high rate of mortality and morbidity.[5] ICH risk is 10–20 times higher in people with hemophilia (PWH) than the general population.[5] Incidence of ICH from select European countries is estimated at 13.9–38.6 per 100,000 males, while the reported incidence in PWHA is 290–540 per 100,000 PWHA.[5]

In a US-based study, 1.9% of the cohort with hemophilia, which included individuals with hemophilia A or B, experienced an ICH, resulting in an incidence rate of 390/100,000 patient-years.[6] Witmer et al used data from the Universal Data Collection project, collected by the Centers for Disease Control and Prevention, from >20,000 people with bleeding disorders from May 1998 through March 2008.[6] This study found that prophylaxis was associated with

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a significant risk reduction for ICH occurrence in people with severe hemophilia who were negative for HIV or inhibitors.[6] Prophylaxis with clotting factor concentrates is currently an established standard of care for people with severe hemophilia A (factor levels <0.01 IU/mL; <1% of normal) and some people with moderate hemophilia A (0.01–0.05 IU/mL; 1–5% of normal).[1,2] FVIII inhibitors can develop in response to treatment with FVIII concentrate and are associated with increased mortality and decreased effectiveness of factor concentrate; however, the effect of inhibitor status on bleeding phenotype is unclear.[7–9]

Risk factors for ICH identified by Witmer et al included being between 2 and 9 years of age or >41 years of age, presence of a high-titer inhibitor, prior ICH, severe hemophilia, and hepatitis C (HCV) infection.[6] A 2011 study of an Italian cohort of PWH also identified an increased risk of ICH in young children, adults aged >40 years, people with severe hemophilia diagnosis, and people with inhibitors.[10] People with hemophilia A or hemophilia B were included in both studies.[6,10]

Since publication of the Witmer study in 2010, the standard of care and treatment landscape for PWHA have changed.[2] Here, we used contemporary ATHNdataset data to investigate risk factors for ICH and identify possible associations between time to developing ICH and various analyzed characteristics in individuals with hemophilia A.

Methods

Study Design

This retrospective cohort study included male (assigned at birth) PWHA in the ATHNdataset, with visit information from January 1, 2010 through September 30, 2020. The American Thrombosis & Hemostasis Network (ATHN) is the steward of the ATHNdataset, a Health Insurance Portability and Accountability Act–compliant, de-identified patient health dataset containing data from individuals with bleeding and clotting disorders receiving care in the United States at ATHN-affiliated treatment centers. Individuals may opt in or consent to contribute their data to help establish a better understanding of blood disorders, including complications, social and economic costs, and the effectiveness of treatments and interventions. With data contributed by >61,000 individuals, ~17,000 of whom have HA, the ATHNdataset is the largest source of health data of PWH in the world.

Only individuals with moderate (FVIII levels 1–5%)/severe (FVIII levels <1%) HA who opted in or consented to enrollment in the ATHNdataset were included in this study. While individuals with factor deficiencies other than HA were not ruled out from this dataset, those with a history of any bleeding disorder as primary diagnosis other than HA were excluded. Individuals with mild HA or with missing FVIII clotting activity levels were also excluded. ICH cases were not discriminated as spontaneous or trauma related. ICH status was based on documentation in real-world medical records, which were extracted anonymously into the ATHNdataset. There is no independent verification of the data in the ATHNdataset.

Demographics, Variables, and Outcome

The variables assessed in this study included race, ethnicity, age, mortality, Medicaid coverage, HCV status, HIV status, hypertension (HTN) status, FVIII treatment (plasma derived [pd] and/or recombinant [r]), bypassing agent treatment, non-factor treatment, prophylaxis status, and inhibitor status. Subgroups for each variable were defined based on relevant criteria. Mortality, assessed at study end, was evaluated for impact on ICH incidence during the study period. History of or current HCV/HIV infection and/or HTN were documented in real-world medical records, which were, in turn, anonymously extracted into the ATHNdataset. Individuals were described as “ever having received prophylaxis” if at any time in their medical history they received prophylaxis, defined as the use of any treatment product on a regular basis to prevent bleeds and/or maintain tolerance to factor. Individuals receiving episodic treatment were classified as never having received prophylaxis. Information on patient clinical data, individual prophylactic regimens related to frequency and dose, or temporally restricted inhibitor positivity and inhibitor titers were unavailable for this study.

The primary outcome of interest was time from first recorded visit in the ATHNdataset to the first incidence of ICH or right-censor (death or end of study period).

Statistical Methods

Unadjusted Analysis

ICH events were evaluated for frequency over the study period by linear regression. Significance was determined by *P*-value of the *F*-test, with a cutoff of 0.05. The association between incidence of ICH and each individual variable was measured in a contingency table analysis. Incidence rates (IRs; reported per 1000 patient-years) were calculated for prophylaxis and inhibitor in subgroup analyses by removing individuals with unknown status for either characteristic. The incidence rate ratio (IRR) was then calculated using the IR values for individuals with or without history of prophylaxis and FVIII inhibitors, respectively. Significance was determined through chi-square test with α=0.05.

Unadjusted univariate Cox proportional-hazards (CoxPH) analysis, which considers time to occurrence and incidence of ICH, was assessed for each subgroup associated with a variable across the cohort. Hazard ratios (HRs) were estimated and plotted for each variable using the CoxPH model against the subgroup reference. Survival curves were plotted for key subgroup variables, and significance was determined by *P*-value of the log rank test, with a cutoff of 0.05.

Multivariable Analyses

All assessed variables were used to develop a multivariable CoxPH model. Three more multivariable CoxPH models were developed: one included only significant covariates from the unadjusted CoxPH analysis, one additionally included inhibitor status (regardless of significance in the unadjusted analysis), and one further incorporated an interaction between inhibitor and prophylaxis. Significance for subgroups associated with a variable was determined using α=0.05 against the reference subgroup for the variable analyzed.

Sensitivity Analyses

Sensitivity analyses were conducted because of the small number of PWHA who experienced ICH in proportion to the cohort and were compared with their respective original models. A stratified bootstrapping model was applied to the unadjusted univariate and multivariable CoxPH models. This approach was done by defining the estimate or coefficient for bootstrapping specific to the model. Three hundred individuals were randomly sampled from the ICH and no-ICH populations and were bootstrapped 1000 times. Bootstrap statistics included a mean of estimates, bias, and confidence interval (CI) and were compared with their respective original model using the full dataset. Three ridge-regression penalties (small: θ=1, medium: θ=5, large: θ=10) were applied to the CoxPH multivariable model to address bias that could occur when the number of events of interest is rare, using all data to generate the model. Significance for subgroups associated with a variable was determined using α=0.05 against the reference subgroup for the variable analyzed.

Results

Patient Characteristics

The initial cohort consisted of 8065 PWHA. Only male (assigned at birth) PWHA aged between 2 and 75 years with and without ICH were included (*n*=7863). PWHA <2 years of age and >75 years of age were excluded because of the lack of comparator individuals within each age group; all 22 PWHA <2 years of age had a documented ICH incident, and none of the 95 PWHA >75 years of age experienced an ICH incident. Sixteen PWHA with ICH from this cohort were excluded from the final analysis because ICH occurrence was before the study period and therefore could not be used to identify risk factors for new-onset ICH. An additional 10 PWHA were excluded from the final analysis because they did not have a visit during the study timeframe (*n*=7837; Supplementary Figure 1). Demographic, clinical, and treatment characteristics of the final cohort are summarized in Table 1. ICH incidence rate significantly decreased during the 10-year study period (coefficient: –19.66; *P*<0.001; Figure 1).

Table 1 Demographic, Clinical, and Treatment Characteristics of the Cohort and Incidence of ICH

Characteristic, n (%)	Total Cohort (N=7837)	No ICH, n (%) (n=7702)	ICH, n (%) (n=135)	*P*-value
Race				0.200
White	5937 (75.8)	5832 (75.7)	105 (77.8)	
Black or African American	1156 (14.8)	1132 (14.7)	24 (17.8)	
Other	610 (7.8)	604 (7.8)	6 (4.4)	
Unknown	134 (1.7)	134 (1.7)	0 (0)	
Ethnicity				0.700
Not Hispanic, Latinx, or Spanish origin	6482 (82.7)	6370 (82.7)	112 (83.0)	
Hispanic, Latinx, or Spanish origin	1314 (16.8)	1292 (16.8)	22 (16.3)	
Unknown	41 (0.5)	40 (0.5)	1 (0.7)	
Age, years				<0.001
2–12	1003 (12.8)	956 (12.4)	47 (34.8)	
13–18	1336 (17.0)	1317 (17.1)	19 (14.1)	
19–29	2221 (28.3)	2205 (28.6)	16 (11.9)	
30–49	2322 (29.6)	2293 (29.8)	29 (21.5)	
50–74	955 (12.2)	931 (12.1)	24 (17.8)	
Mortality				0.011
Alive	7588 (96.8)	7463 (97.3)	125 (92.6)	
Deceased	249 (3.2)	239 (3.1)	10 (7.4)	
Current or prior Medicaid				0.018
No	4778 (61.0)	4709 (61.1)	69 (51.1)	
Yes	3059 (39.0)	2993 (38.8)	66 (48.9)	
Current or prior HCV				0.011
Yes with known start date	463 (5.9)	447 (5.8)	16 (11.9)	
Yes with unknown start date	803 (10.2)	792 (10.2)	11 (8.1)	
No	6571 (83.8)	6463 (83.9)	108 (80.0)	
Current or prior HIV				<0.001
Yes with known start date	384 (4.9)	367 (4.8)	17 (12.6)	
Yes with unknown start date	335 (4.3)	328 (4.3)	7 (5.2)	
No	7118 (90.8)	7007 (91.0)	111 (82.2)	
Current or prior HTN				0.001
Yes with known start date	56 (0.7)	53 (0.7)	3 (2.2)	
Yes with unknown start date	143 (1.8)	135 (1.8)	8 (5.9)	
No	7638 (97.5)	7514 (97.6)	124 (91.9)	

(*Continued*)

Table 1 (Continued).

Characteristic, n (%)	Total Cohort (N=7837)	No ICH, n (%) (n=7702)	ICH, n (%) (n=135)	*P*-value
Current or prior rFVIII				<0.001
Yes with known start date	6334 (80.8)	6235 (81.0)	99 (73.3)	
Yes with unknown start date	785 (10.0)	777 (10.1)	8 (5.9)	
No	718 (9.2)	690 (9.0)	28 (20.7)	
Current or prior pdFVIII				0.034
Yes with known start date	883 (11.3)	859 (11.2)	24 (17.8)	
Yes with unknown start date	126 (1.6)	123 (1.6)	3 (2.2)	
No	6828 (87.1)	6720 (87.3)	108 (80.0)	
Current or prior FVIII (rFVIII and/or pdFVIII)				0.001
Yes with known start date	6612 (84.4)	6504 (84.4)	108 (80.0)	
Yes with unknown start date	793 (10.1)	782 (10.2)	11 (8.1)	
No	433 (5.5)	416 (5.4)	17 (12.6)	
Current or prior bypassing agent				0.007
Yes with known start date	601 (7.7)	581 (7.5)	20 (14.8)	
Yes with unknown start date	77 (1.0)	75 (1.0)	2 (1.5)	
No	7159 (91.3)	7046 (91.5)	113 (83.7)	
Current or prior non-factor treatment				<0.001
Yes with known start date	1878 (24.0)	1872 (24.3)	6 (4.4)	
Yes with unknown start date	145 (1.9)	144 (1.9)	1 (0.7)	
No	5814 (74.2)	5686 (73.8)	128 (94.8)	
Current or prior prophylaxis				0.120
Yes	5647 (72.1)	5559 (72.2)	88 (65.2)	
No	1550 (19.8)	1514 (19.7)	36 (26.7)	
Yes with unknown start date	640 (8.2)	629 (8.2)	11 (8.1)	
Current or prior inhibitor				0.200
Yes	434 (5.5)	422 (5.5)	12 (8.9)	
No	6665 (85.0)	6557 (85.1)	108 (80.0)	
Yes with unknown start date	738 (9.4)	723 (9.4)	15 (11.1)	

Abbreviations: FVIII, factor VIII; HCV, hepatitis C virus; HTN, hypertension; ICH, intracranial hemorrhage; pdFVIII, plasma-derived FVIII; rFVIII, recombinant FVIII.

Incidence of ICH

Unadjusted contingency table analysis was performed to test the association between incidence of ICH and each variable (Table 1). Because of small sample size, pdFVIII and rFVIII treatments were combined (FVIII combined). Bypassing agent and non-factor treatment parameters were not further analyzed because of low utilization rates in the study cohort.

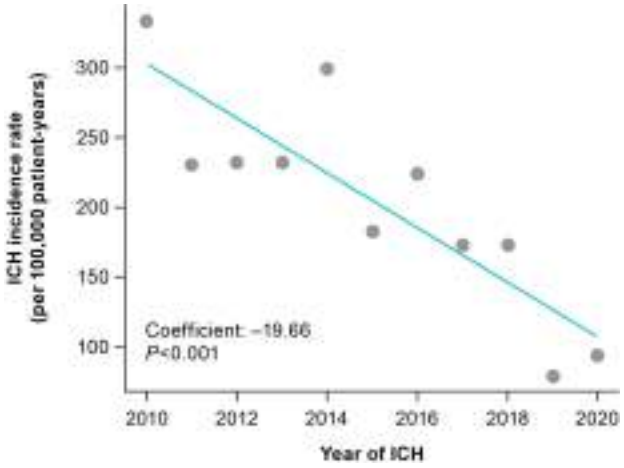

Figure 1 ICH incidence rate during the study period (2010–2020).
Abbreviation: ICH, intracranial hemorrhage.

Over 10 years and 9 months of collected data, 135 of 7837 PWHA (1.7%) had an ICH, with a median follow-up time of 10.7 patient-years. ICH incidence was found to be dependent on age (P<0.001), Medicaid status (P=0.018), mortality status (P=0.011), HCV status (P=0.011), HIV status (P<0.001), HTN status (P=0.001), and combined FVIII treatment status (P=0.001) and not found not to be dependent on race (P=0.200) or ethnicity (P=0.700). No dependence was found between incidence of ICH and prophylactic treatment (P=0.120) or ICH and inhibitor status (P=0.200).

IRR by prophylactic status (0.63; 95% CI, 0.43–0.94) was significant (P=0.020), showing increased incidence of ICH among individuals never receiving prophylaxis; the IR for individuals receiving prophylaxis and not receiving prophylaxis was 162 and 255 per 100,000 patient-years, respectively. IRR by inhibitor status (1.76; 95% CI, 0.97–3.20) was nonsignificant (P=0.059) but showed a numerically increased incidence of ICH among individuals with inhibitors; IR for individuals with and without inhibitors was 303 and 172 per 100,000 patient-years, respectively. No significant associations were found in the five age groups when stratified by prophylaxis status (Supplementary Table 1).

Most significant variables in the contingency table analysis remained significant in the unadjusted univariate CoxPH analysis, which provided additional insights into differential ICH risk in analyzed subgroups (Figure 2). Covariates with a significant protective effect on ICH risk (HR <1) include age >12 years (P<0.001) and no prior HCV (P=0.011), HIV (P<0.001), or HTN (P=0.049; Figure 3); availability of a start date for the three assessed comorbidities had no apparent bearing on ICH risk relative to having a history of that comorbidity. Past/current absence of an inhibitor was found not to have a significant effect on ICH risk (P=0.067; Figure 3) but showed a numerically decreased HR. Covariates indicating a significantly greater ICH risk (HR >1) include mortality after an ICH (P=0.010), current or past Medicaid coverage (P=0.017), and absence of pdFVIII or rFVIII treatment (P<0.001; Figure 3). Individuals who had never received prophylactic treatment were found to carry significantly greater risk of developing ICH than individuals receiving prophylactic treatment (HR 1.56; 95% CI, 1.06–2.30; P=0.024; Figure 2). Neither race nor ethnicity had a significant impact on risk of developing ICH (Figure 2).

Kaplan–Meier plots largely corroborated the unadjusted univariate CoxPH HR analyses (Figure 3); however, there were only marginal differences in the survival curves for the three prophylaxis subgroups (P=0.074). Bootstrapping sensitivity analysis of the unadjusted univariate CoxPH model analysis corroborated significance and directionality of most key characteristics; however, never having had HCV or HTN no longer had a significant protective effect on ICH development (Supplementary Table 2).

Multivariable Analysis

Multivariable CoxPH survival analysis with all variables was largely consistent with previous contingency table and univariate analyses. Although the effect of never receiving prophylaxis was found to be nonsignificant (P=0.070), the HR was numerically increased when compared with the reference group (Supplementary Figure 2). The risk of developing

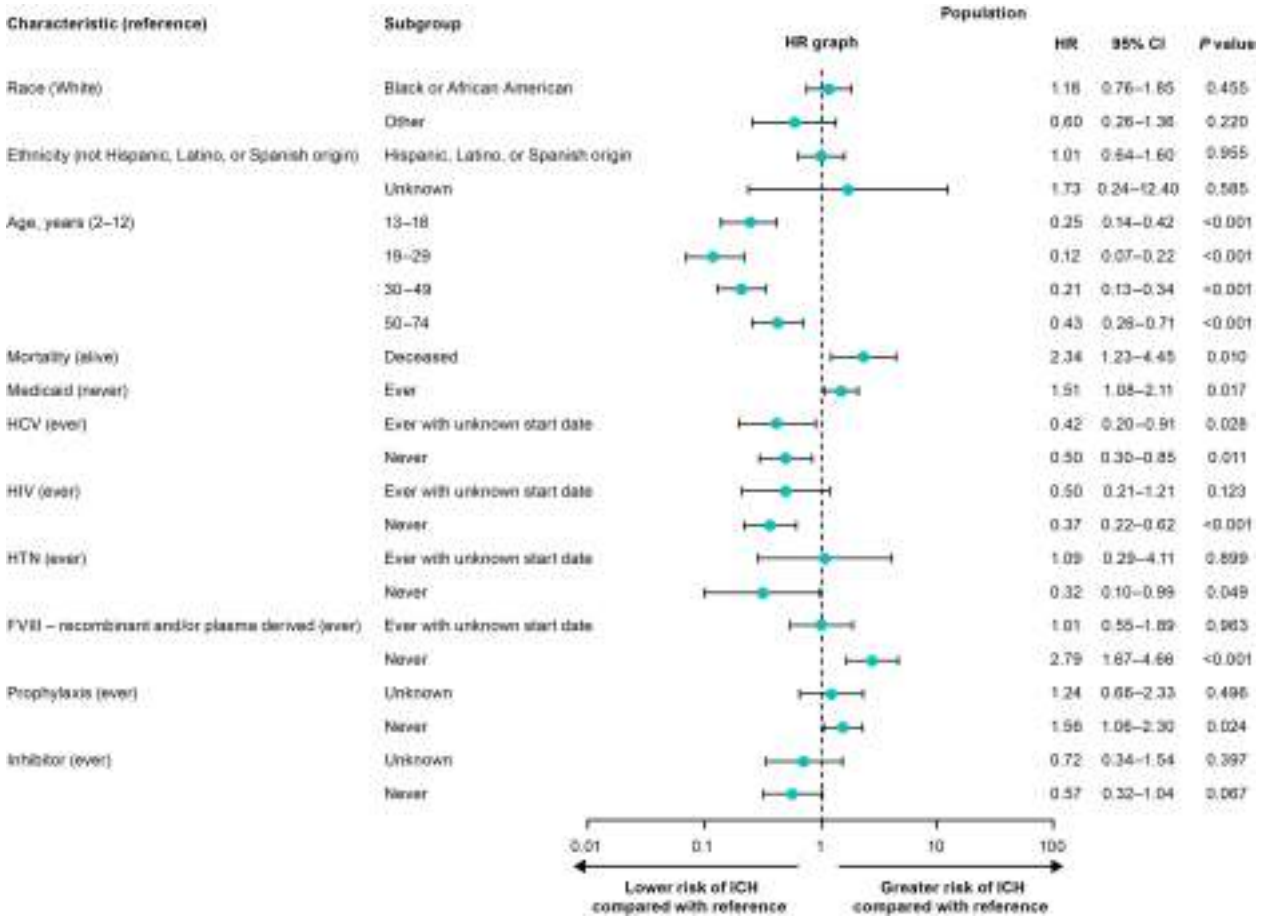

Figure 2 Risk of ICH in univariate analysis.
Abbreviations: CI, confidence interval; FVIII, factor VIII; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, hazard ratio; HTN, hypertension; ICH, intracranial hemorrhage.

ICH was also not significantly associated with a negative history of HCV (P=0.06) or with mortality in this analysis (P=0.257; Supplementary Figure 2).

Two additional multivariable CoxPH models were generated, one including only covariates found to be significant in the unadjusted population CoxPH analysis and another also including inhibitor status (Supplementary Figure 3; Supplementary Figure 4). In the former, never having received prophylaxis (P=0.064) and never having had HCV (P=0.072) were both nonsignificant, although HR directionality matched previous analyses (Supplementary Figure 3). In the latter, similar results were observed in subgroups that never received prophylaxis (P=0.060) and never had HCV (P=0.071); never having had HTN was also found to be nonsignificant (P=0.051), with a numerically decreased HR compared with the reference group (Supplementary Figure 4).

Finally, multivariable CoxPH analysis, built using all significant covariates from the unadjusted analysis, inhibitor status, and with a prophylaxis:inhibitor interaction, was considered (Figure 4). Although most of the covariates were consistent in both significance and directionality of effect on ICH with the previously analyzed model, the HR for never having received prophylactic treatment increased exponentially to 7.67, while never having had HCV was found to be nonsignificant (P=0.098; Figure 4). This analysis also considered the joint impact of prophylaxis and inhibitor status on ICH risk; compared with the reference group (having had inhibitors [with unknown start date] and having received prophylaxis [with unknown start date]), never having received prophylaxis and never having had inhibitors, jointly, was significantly protective against ICH risk (P=0.005; Figure 4).

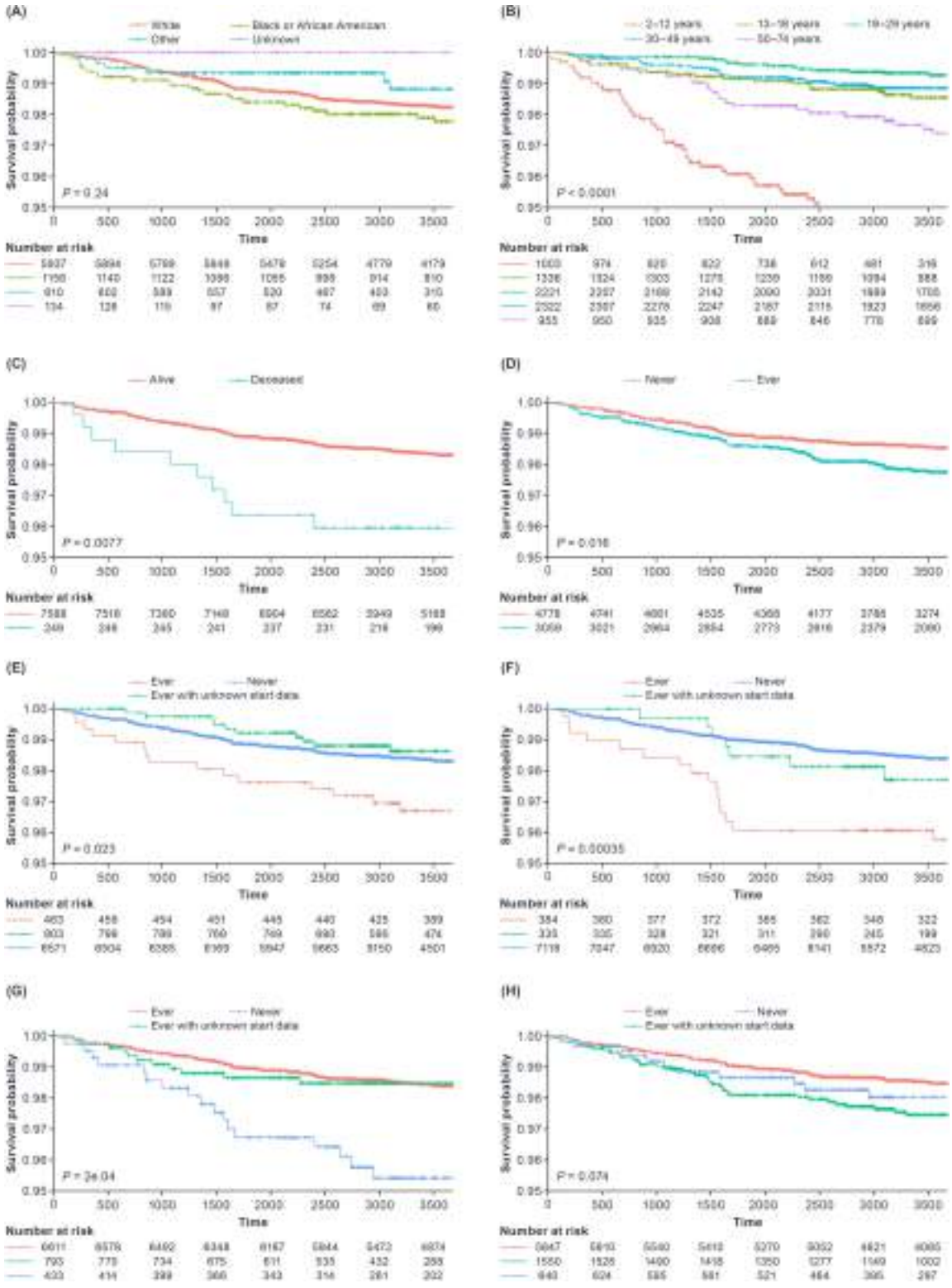

Figure 3 Kaplan–Meier curves of key characteristics. (**A**) Race; (**B**) Age; (**C**) Mortality; (**D**) Medicaid; (**E**) Hepatitis C; (**F**) HIV; (**G**) Any FVIII treatment (pdFVIII and rFVIII); (**H**) Prophylaxis.

Abbreviations: FVIII, factor VIII; HCV, hepatitis C virus; HIV, human immunodeficiency virus; pdFVIII, plasma-derived factor VIII; rFVIII, recombinant factor VIII.

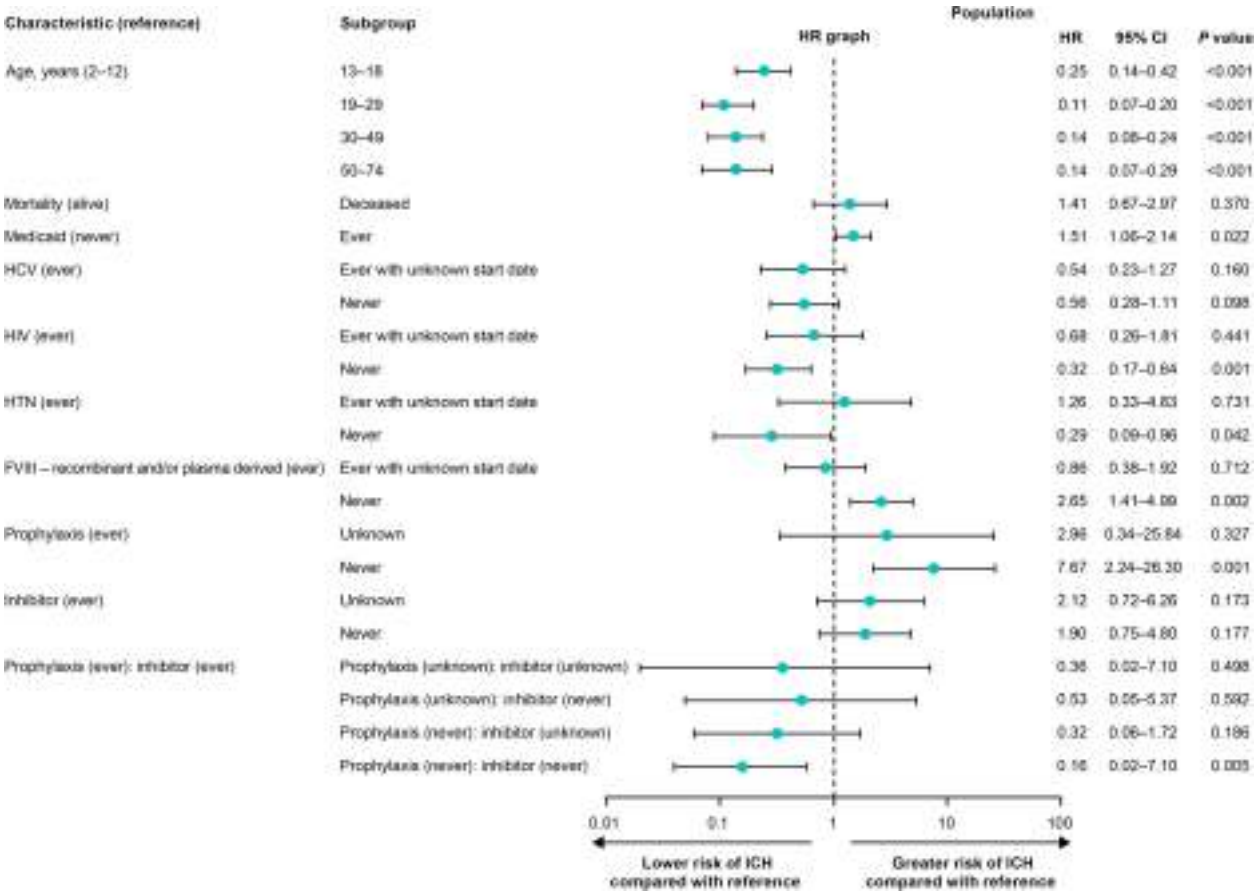

Figure 4 Risk of ICH in multivariable analysis with significant covariates (univariate analysis) and prophylaxis:inhibitor interaction.
Abbreviations: CI, confidence interval; FVIII, factor VIII; HCV, hepatitis C virus; HR, hazard ratio; HIV, human immunodeficiency virus; HTN, hypertension; ICH, intracranial hemorrhage.

Bootstrapping and penalty-based ridge-regression sensitivity analyses were conducted on a multivariable model with interaction to confirm results (Table 2 and Table 3). Overall, the bootstrapping analysis minimized HR estimates; however, directionality and significance were largely maintained compared with the original model (Table 2). Based on the estimated 95% CI, not having had HTN no longer had a significant protective effect on ICH occurrence. The HR

Table 2 Multivariable CoxPH Model with Interaction and Bootstrap Sensitivity Analyses

Characteristic	Population		Bootstrap Sensitivity	
	HR	95% CI	HR	95% CI
Age, years (reference: 2–12)				
13–18	0.25	0.14–0.42	0.38	0.23–0.50
19–29	0.11	0.065–0.20	0.24	0.12–0.27
30–49	0.14	0.077–0.24	0.25	0.12–0.28
50–74	0.14	0.066–0.29	0.22	0.10–0.34

(*Continued*)

Table 2 (Continued).

Characteristic	Population		Bootstrap Sensitivity	
	HR	95% CI	HR	95% CI
Mortality (reference: alive)				
Deceased	1.41	0.67–2.97	2.03	0.87–3.53
Current or prior Medicaid (reference: no)				
Yes	1.51	1.06–2.14	1.15	0.99–1.67
Current or prior HCV (reference: yes)				
Yes with unknown start date	0.54	0.23–1.27	0.33	0.21–0.89
No	0.56	0.28–1.11	0.44	0.29–0.92
Current or prior HIV (reference: yes)				
Yes with unknown start date	0.68	0.26–1.81	0.90	0.34–1.99
No	0.32	0.17–0.64	0.39	0.24–0.79
Current or prior HTN (reference: yes)				
Yes with unknown start date	1.26	0.33–4.83	0.81	0.22–2.69
No	0.29	0.088–0.96	0.47	0.11–1.05
FVIII (rFVIII and/or pdFVIII; reference: yes)				
Yes with unknown start date	0.86	0.38–1.92	2.01	0.00–3.63
No	2.66	1.41–4.99	2.12	1.03–3.06
Current or prior prophylaxis (reference: yes)				
Yes with unknown start date	2.96	0.34–25.84	0.74	0.00–3.63
No	7.67	2.24–26.30	2.03	1.30–9.12
Current or prior inhibitor (reference: yes)				
Yes with unknown start date	2.12	0.72–6.26	1.08	0.64–3.39
No	1.90	0.75–4.80	1.04	0.73–3.03
Current or prior prophylaxis, inhibitor interaction (reference: yes [with unknown start date], yes [with unknown start date])				
Prophylaxis (no) Inhibitor (no)	0.16	0.044–0.58	0.59	0.12–0.92
Prophylaxis (yes with unknown start date) Inhibitor (no)	0.53	0.053–5.37	1.08	0.33–2.6e6
Prophylaxis (no) Inhibitor (yes with unknown start date)	0.32	0.061–1.72	0.59	0.15–2.83
Prophylaxis (yes with unknown start date) Inhibitor (yes with unknown start date)	0.36	0.018–7.10	1.27	0.00–1.7e6

Abbreviations: CI, confidence interval; CoxPH, Cox proportional hazards; FVIII, factor VIII; HCV, hepatitis C virus; HR, hazard ratio; HTN, hypertension; pdFVIII, plasma-derived FVIII; rFVIII, recombinant FVIII.

Table 3 Multivariable CoxPH Model with Interaction and Penalty-Based Ridge-Regression Analyses

Characteristic	Population		RR1		RR2		RR3	
	HR	*P*-value	HR	*P*-value	HR	*P*-value	HR	*P*-value
Age, years (reference: 2–12)								
13–18	0.25	<0.001	0.26	<0.001	0.30	<0.001	0.34	<0.001
19–29	0.12	<0.001	0.12	<0.001	0.15	<0.001	0.18	<0.001
30–49	0.14	<0.001	0.15	<0.001	0.18	<0.001	0.23	<0.001
50–74	0.14	<0.001	0.15	<0.001	0.20	<0.001	0.26	<0.001
Mortality (reference: alive)								
Deceased	1.41	0.370	1.41	0.365	1.42	0.351	1.42	0.337
Current or prior Medicaid (reference: no)								
Yes	1.51	0.022	1.51	0.022	1.51	0.019	1.50	0.018
Current or prior HCV (reference: yes)								
Yes with unknown start date	0.54	0.160	0.54	0.155	0.55	0.151	0.57	0.150
No	0.56	0.098	0.58	0.108	0.63	0.157	0.68	0.219
Current or prior HIV (reference: yes)								
Yes with unknown start date	0.68	0.441	0.69	0.448	0.70	0.462	0.72	0.477
No	0.33	0.001	0.34	0.001	0.37	0.002	0.40	0.004
Current or prior HTN (reference: ever)								
Ever with unknown start date	1.26	0.731	1.27	0.727	1.28	0.716	1.29	0.698
Never	0.29	0.042	0.30	0.046	0.33	0.059	0.35	0.073
Current or prior FVIII (rFVIII and/or pdFVIII; reference: yes)								
Yes with unknown start date	0.86	0.712	0.86	0.713	0.88	0.739	0.89	0.758
No	2.65	0.002	2.66	0.002	2.62	0.002	2.55	0.002
Current or prior prophylaxis (reference: yes)								
No	7.67	0.001	5.99	0.002	4.11	0.007	3.09	0.018
Yes with unknown start date	2.96	0.327	2.32	0.420	1.67	0.578	1.40	0.679
Current or prior inhibitor (reference: yes)								
No	1.90	0.177	1.64	0.246	1.33	0.436	1.17	0.645
Yes with unknown start date	2.12	0.173	1.82	0.238	1.46	0.402	1.27	0.565
Current or prior prophylaxis, inhibitor interaction (reference: yes [with unknown start date], yes [with unknown start date])								
Prophylaxis (no) Inhibitor (no)	0.16	0.005	0.21	0.009	0.30	0.028	0.39	0.068

(*Continued*)

Table 3 (Continued).

Characteristic	Population		RR1		RR2		RR3	
	HR	*P*-value	HR	*P*-value	HR	*P*-value	HR	*P*-value
Prophylaxis (yes with unknown start date) Inhibitor (no)	0.53	0.592	0.68	0.729	0.92	0.934	1.08	0.935
Prophylaxis (no) Inhibitor (yes with unknown start date)	0.32	0.186	0.41	0.273	0.57	0.467	0.72	0.666
Prophylaxis (yes with unknown start date) Inhibitor (yes with unknown start date)	0.36	0.498	0.46	0.596	0.63	0.743	0.75	0.830

Abbreviations: CoxPH, Cox proportional hazards; FVIII, factor VIII; HCV, hepatitis C virus; HR, hazard ratio; HTN, hypertension; pdFVIII, plasma-derived FVIII; rFVIII, recombinant FVIII; RR1, ridge regression penalty 1; RR2, ridge regression penalty 2; RR3, ridge regression penalty 3.

of not having received prophylactic treatment decreased from 7.67 to 2.03 following bootstrapping analysis but was significant based on the estimated CI.

Ridge-regression penalties were also applied to the multivariable CoxPH model with a prophylaxis:inhibitor interaction; as expected, smaller penalties impacted HR estimates less than larger penalties (Table 3). Compared with the original model, mortality and HCV remained nonsignificant, and HTN status went from significant to nonsignificant. Penalty-based ridge regression minimized HR estimates while maintaining directionality and significance compared with the population-based multivariable CoxPH model with an interaction term for other variables. The HR of never having received prophylactic treatment decreased from 7.67 to 3.09 following application of the largest ridge-regression penalty.

Discussion

ICH is a serious complication for PWH. Identifying factors associated with increased ICH risk is critical for its prevention. Our study identified the following risk factors for ICH in PWHA using the ATHNdataset: being between 2 and 12 years of age, having ever received Medicaid coverage, having had certain comorbidities (HIV and HTN), never having received factor treatment, and never having received prophylactic treatment.

This is the first US study in over a decade to identify risk factors for ICH and associations between risk factors and time to developing ICH. Witmer et al reported that any of the following conditions confer significant risk of ICH: age 2–9 or >41 years, being of Black (non-Hispanic) descent, and having HCV, high-titer inhibitors, severe hemophilia, or prior ICH.[6] A significant interaction between prophylaxis and both inhibitor and HIV status could only be found in people with severe hemophilia, not in the entire cohort; absence of an inhibitor and no documented HIV infection were found to be protective against development of ICH.[6,10]

Since the Witmer study, the standard of care and the overall hemophilia treatment landscape have evolved, necessitating our follow-up study using more recent ATHNdataset data.[2] Additionally, the Witmer study population included those with either hemophilia A or B; therefore, the findings in that study are not specific to either type.[6] Our study looks at risk factors for ICH specifically in individuals with moderate/severe hemophilia A.

Results from multivariable analysis with an interaction term indicated being aged 13–74 years or never having had HIV were associated with lower risk of ICH, while ever being covered by Medicaid or never having received either FVIII treatment or prophylactic treatment were associated with greater risk of ICH during the study period. These results were largely consistent in directionality and significance following sensitivity analyses. Mortality after study end was found to increase ICH risk, and never having HTN or HCV was found to decrease ICH risk only in the univariate analyses.

Our findings are in agreement with other real-world studies.[5,6,10–14] Hypertension is a known risk factor for ICH in the broad population and was the most common comorbidity among adult PWHA with ICH.[11,15] Prevalence of HTN was higher in PWHA versus those without, especially among older individuals.[11] While HTN was found to be significant only in the univariate analysis, further analysis of ICH cases by age and hypertensive status may illuminate a role in mediating ICH risk. Mortality caused by ICH is higher in PWH when compared with the general population (estimated at 20%) and

is yet higher in young children and in developing countries, emphasizing the need to better understand risk factors for ICH.[5,14] In our study, mortality at study's end was found to be significant only in the univariate analysis and was assessed as a risk factor for ICH, not an outcome.

Analyses from this study demonstrate that PWHA who have ever been covered by Medicaid have a higher risk of developing an ICH. Therefore, Medicaid-insured PWHA could represent an important population for ICH risk mitigation. Medicaid is a public health insurance option in the United States.[16] It has previously been shown that people with Medicaid insurance have worse outcomes after ICH than privately insured individuals;[12] whether this disparity in outcomes exists in Medicaid-insured PWHA requires further study. Given that Medicaid insurance is linked to socioeconomic status, this finding could be further suggestive of an existing health disparity by income and a barrier to achieving health equity.[16,17] Socioeconomic status was not fully captured in the ATHNdataset, opening the possibility that the impact of Medicaid insurance on ICH incidence could be explained by disparities in health equity. Intriguingly, this analysis found no effect of race or ethnicity, both notable contributors to health inequity, on ICH incidence,[18] suggesting that health inequity, alone, does not fully describe the factors contributing to ICH incidence.

Inhibitor status was not found to be a significant risk factor for ICH in this study, but previous studies have demonstrated that presence of inhibitors, particularly high-titer inhibitors, significantly increases ICH risk.[6,10] This could be related to the changing landscape of immune tolerance induction. Over time, variations on the original Bonn protocol and other novel immune tolerance induction regimens have been used to treat persons with FVIII inhibitors.[19] Synchronously, a wider breadth of hemophilia treatments, including extended–half-life factor products and emicizumab, have also been available, although their respective effectiveness in immune tolerance induction has not been definitively established.[20] Altogether, these advances may enable increased use of immune tolerance induction, which may, in turn, preclude establishing a statistically significant link between ICH incidence and inhibitor status.

Previous studies have also demonstrated the ICH protective effect of prophylactic treatment in people with severe hemophilia[6] and in children and adolescents with hemophilia A, corroborating findings from this study.[13] As of 2018, the standard of care for congenital hemophilia A has evolved to include emicizumab, a humanized antibody that mimics activated FVIII to allow continuation of the coagulation cascade.[21] This study's period includes data from before and after emicizumab approval in 2018. The ICH rate significantly decreased across the study period, but data from the periods before and after emicizumab approval cannot be conclusively analyzed because of the limited sample size after emicizumab approval and the inability to account for all possible confounders (Figure 1).

As in the Witmer study,[6] results are only generalizable to people receiving specialized care at hemophilia treatment centers (HTCs). In a study from 1998, HTCs were estimated to treat 60–70% of PWH in the United States; however, there are no data to confirm this.[22] Newer data suggest that close to 80% of PWH are treated at HTCs.[3] The ATHNdataset, which contains data from all ATHN-affiliated treatment centers, only contains information from people who consent or opt in to contribute their data. Therefore, these data may not be generalizable to all PWHA receiving care through the United States HTC Network or the broader population of PWHA in the United States.

Adherence to a prophylactic regimen can dictate whether PWHA experience improved outcomes.[2] In a 2010 survey of US HTC nurses, 80% of people with severe HA were found to be adherent to their prescribed prophylactic regimen. Data related to prophylaxis adherence were not available for the people in this study; however, the inclusion of non-compliant individuals or individuals with a less rigorous prophylactic regimen could lead to an underestimation of the effect of prophylaxis on occurrence of ICH. This suggests that prophylactic treatment may have a more protective effect on risk of developing ICH than assessed in this study.

Although only participants with moderate or severe HA were included in this study, hemophilia severity data were not analyzed because of suspected interactions with clinical and treatment characteristics. Severe hemophilia is a known risk factor for ICH, and prophylaxis is the standard of care for severe hemophilia.[2,6] In a French survey using data from 1991 through 2001, two-thirds of ICH cases were in people with severe hemophilia, and prophylaxis was associated with improved ICH-associated outcomes in PWH.[2,23]

This analysis did not distinguish individuals who have active HIV or HCV infections, or currently have HTN or FVIII inhibitors, from those who have previously had but do not currently have either comorbidity. This will cause an

underestimation of the effect of either HCV, HIV, HTN, or inhibitor status on development of ICH, suggesting that not having either comorbidity may be more protective against ICH occurrence than assessed.

While the Witmer study found a relationship between prophylaxis and inhibitor status in people with severe hemophilia, in our study prophylaxis:inhibitor subgrouping characteristics and sample sizes prevent strong conclusions regarding the joint risk of prophylaxis and inhibitor status on ICH, with the notable exception of inhibitor-negative individuals who have never received prophylaxis.[6]

Conclusions

This study, using data from the ATHNdataset, identified the following risk factors for ICH in PWHA: being aged between 2 and 12 years, having ever received Medicaid coverage, having had certain comorbidities (HIV and HTN), never having received factor treatment, and never having received prophylactic treatment. These risk factors will need to be continually reevaluated as the treatment landscape for hemophilia evolves to include increased use of non-factor products and gene therapy.

Data Sharing Statement

The data supporting the findings of this study originate from the ATHNdataset and are available from ATHN. Restrictions apply to the availability of these data, which were used under license for this study. Data inquiries can be made by emailing ATHN at support@athn.org.

Ethics Statement

The ATHNdataset is a Health Insurance Portability and Accountability Act–compliant, de-identified patient health dataset containing data from individuals with bleeding and clotting disorders receiving care in the United States at ATHN-affiliated treatment centers who have consented to contribute their data voluntarily. Per ethics review by Advarra (www.advarra.com), the ATHNdataset has been deemed non-human research.

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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 of 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.

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Disclosure

JC, MD, AGM, and SX are currently employees and stockholders of Takeda. BE was an employee of Takeda at the time of the study and is currently a stockholder of Takeda. MR was affiliated with American Thrombosis and Hemostasis Network at the time of the study and receives research funding from Bayer, BioMarin, CSL Behring, Genentech, Grifols, Hema Biologics, LFP, NovoNordisk, Octapharma, Pfizer, Sanofi, Sparke, Takeda, and uniQure; is a consultant for BioMarin, Catalyst Biosciences, CSL Behring, Genentech, Hema Biologics, Kedrion, NovoNordisk, Pfizer, Sanofi, Takeda, and uniQure; and is on the board of directors of the Foundation of Women and Girls with Blood Disorders,

Partners in Bleeding Disorders, and the Thrombosis and Hemostasis Societies of North America. The authors report no other conflicts of interest in this work.

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ORIGINAL RESEARCH

Cardiovascular Consequences of Sickle Cell Disease

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Background: Sickle cell disease is an inherited blood disorder which can lead to severe complications, particularly in the cardiovascular and respiratory systems, potentially resulting in arrhythmias, pulmonary hypertension (PH), and cardiomegaly. This study aims to investigate the risk of PH and arrhythmias in adult SCD patients.

Methods: Retrospective analysis of medical records from King Abdulaziz University Hospital (KAUH) for patients with SCD aged 15 and above between 2009 and 2021. The study included 517 patients, with echocardiograms and electrocardiograms assessed according to the European Society of Cardiology/the European Respiratory Society (ESC/ERS) guidelines for categorizing PH risk (low, moderate, high) and detecting arrhythmias. Data analysis employed the Statistical Package for the Social Sciences (SPSS), utilizing quantitative and qualitative data representation. Multivariate logistic regression identified independent risk factors with odds ratios at a 95% confidence interval (CI).

Results: Among participants, 50.3% were male, with a total sample average age of 34.45 ± 9.28 years. Results indicated that 1.4% of patients experienced arrhythmias, 3.7% had a moderate PH risk, and 3.3% were classified as high PH risk. Logistic regression revealed significant independent risk factors for PH and arrhythmia in patients with SCD, with chronic kidney disease (CKD) carrying the highest odds (26.4 times higher odds of PH and 15.36 times higher odds of arrhythmias).

Conclusion: Patients with SCD are at risk for developing PH and various arrhythmias but are often underdiagnosed. Key risk factors for PH included CKD, liver cirrhosis, and pre-existing cardiac conditions. Arrhythmias were significantly associated with CKD and pre-existing cardiac conditions. To mitigate these risks, we recommend involving a multidisciplinary healthcare team in the care of adult patients with SCD. Future prospective studies are advised for early detection of PH and arrhythmias in hemoglobinopathy patients, potentially reducing mortality.

Keywords: sickle cell disease, arrhythmia, pulmonary hypertension, chronic kidney disease, hemoglobinopathies, PH risk

Introduction

Sickle cell disease (SCD) is a group of genetic red blood cell disorders, inherited as an autosomal recessive condition characterized by the presence of an abnormal hemoglobin S leading to polymerization of hemoglobin in the deoxygenated state causing changes and distortion in the shape of red blood cells and reduction of the flexibility of the hemoglobin.[1–4] Sickle cell disease has two major components participating and playing a major role in most of the complications related to SCD: vaso-occlusive crises and hemolysis.[2,5] During the inflammatory state, the interaction that happens between these distorted red blood cells with the white blood cells and the endothelium will lead to adhesion of this complex and will result in the occlusion of the blood vessels causing ischemia to different vital organs, including blood vessels supplying the cardio-respiratory and the cardio-vascular systems.[6] Sickle cell disease in general involves many other subgroups under its umbrella, including sickle cell anemia, where the only abnormality is the presence of hemoglobin S; hemoglobin SC disease, where the hemoglobin S is

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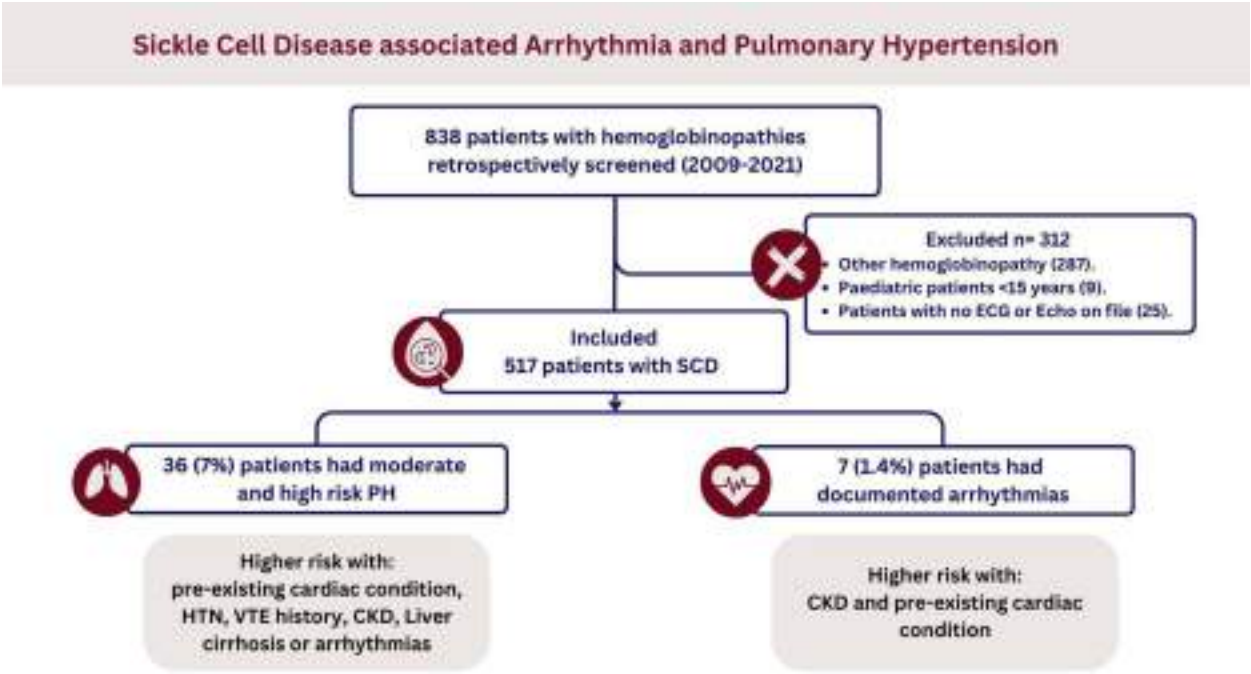
Graphical Abstract



presented with another mutation in the beta-globin chain of the hemoglobin forming hemoglobin C; and hemoglobin sickle beta thalassemia, as the patient is carrying both mutation of hemoglobin S and the mutation of beta thalassemia at the same time.[5] Since the first description of SCD more than a hundred years ago, our understanding of this inherited disease has dramatically improved and tis reflected in a positive way in patient care and management[5,7,8] This evolution started in 1984 with the first reported utilization of hydroxyurea in SCD patients to increase the level of fetal hemoglobin and decrease the crises and complications related to the disease[9] This evolution has continued in the last 10 years by introducing new agents such as voxelotor, crizanlizumab, L-glutamin,[10,11] and, most recently, gene therapy[12] The adoption of some guidelines and methods in the care and management of SCD, such as vaccination and intensive screening, to address different expected complications has significantly decreased morbidity and mortality.[1,13] A big difference is now noticed in morbidity and mortality between high income and low income countries, as training of healthcare providers and access to services are much more easily provided in high income countries. These disorders may also cause fatal complications, especially cardiopulmonary issues such as arrhythmias, tachycardia, pulmonary hypertension (PH), and cardiomegaly. Pulmonary hypertension is a pathophysiological condition with variable clinical presentations in the early and late stages of multiple clinical conditions, which can affect directly or indirectly the respiratory and cardiovascular systems.[3,14] It is defined as a resting mean pulmonary artery pressure (mPAP) of ≥25 mmHg, with a mean pulmonary artery wedge pressure (PAWP) or left ventricular end diastolic pressure (LVEDP) of ≤15 mm Hg plus increased pulmonary vascular resistance (PVR).[3] PH is of great importance and should be viewed with special consideration in patients with SCD as it is relatively common in those patients, and has significant implications for morbidity and mortality[8] The early symptoms of pulmonary hypertension in sickle cell patients are usually non-specific and do not differ from symptoms presented in SCD with no pulmonary hypertension.[14,15] Pulmonary hypertension is usually confirmed by right heart catheterization, but there are some limitation of using this method in all suspected cases of pulmonary hypertension, as it is too expensive, is an invasive procedure, and is not available and accessible everywhere. Other non-invasive methods such as Doppler echo and NT-pro-BNP, which are not accurate enough to diagnose pulmonary hypertension and do not replace right side catheterization, are acceptable as alternative methods to identify and screen the group of patients at high risk of developing pulmonary hypertension.[16] Pulmonary hypertension is classified into five general

groups: pulmonary arterial hypertension, pulmonary hypertension due to left heart disease, pulmonary hypertension due to lung disease and / or hypoxia, chronic thrombo-embolic pulmonary hypertension, and pulmonary hypertension with unclear or multifactorial mechanisms. Pulmonary hypertension related to SCD and hemolysis was initially classified under the category of pulmonary arterial hypertension in the published guidelines in 2009, which was written by the task force for treatment and diagnosis of pulmonary hypertension, a collaborative work between the European Respiratory Society and the European Society of Cardiology.[17] The classification of pulmonary hypertension associated with SCD and hemolysis was then changed in 2013 at the Fifth World Symposium on Pulmonary Hypertension, and is now classified under the category of pulmonary hypertension with unclear or multiple etiology.[18] Depending on the hemodynamics of the cardio-respiratory circulation, pulmonary hypertension can be subdivided into two major categories: precapillary and post-capillary pulmonary hypertension. The subtype is usually recognized and categorized during the procedure of right heart catheterization locoing for and calculating two variables: pulmonary capillary wedge pressure and left ventricular end diastolic pressure. If one of these two variables is greater than 15, pulmonary hypertension will be categorized as post-capillary pulmonary hypertension; if one of the two variables is less than or equal to 15, it will be considered to be precapillary pulmonary hypertension. In SCD patients, there is an equal distribution between precapillary and post-capillary pulmonary hypertension among those who have pulmonary hypertension.[19] Several risk factors were identified to contribute to the pathogenesis of PH in patients with SCD, including ongoing hemolysis and hypoxic pulmonary vasoconstriction, microvascular occlusion, left ventricle dysfunction as well as a chronic inflammatory state, decreased nitrous oxide and hypercoagulability. In SCD patients, any oxidatively stressed environment can lead to the production of a reactive oxygen species (ROS), which can damage the endothelium; this ROS is found to be inhibited by nitrous oxide synthase. A decreased level of nitric oxide can enhance red blood cell adhesion to the endothelium.[20,21] With regard to cardiac arrhythmias, it is defined as irregularities in the rhythm of the heartbeat, which could be either bradycardia or tachycardia; it affects all age groups. With recent advances in understanding the underlying electrophysiology of the heart and the development of arrhythmias, two major mechanisms of arrhythmias have been identified: enhanced or abnormal impulse formation or conduction disturbances.[22]

A study conducted in the United States concluded that the prevalence of PH among patients with SCD was approximately 30%, subcategorized into 17% mild, 8% moderate, and 3% severe. The prevalence was noticed to be higher in the group of SCD patients who had higher fetal hemoglobin level and lower systolic blood pressure.[23] This is in keeping with another single-center study, conducted in Saudi Arabia, where prevalence was found to be 38%, with most being sub-categorized as mild PH; higher prevalence was noticed in the group of SCD patients who had a higher serum ferritin level and a lower fetal hemoglobin level.[24] Studies that performed right heart catheterization for patients with elevated peak tricuspid regurgitant jet velocity reported a confirmed diagnosis of PH in 10% of patients with SCD. Higher prevalence was demonstrated in those patients with SCD who had a lower hematocrit level, and higher levels of direct bilirubin, aspartate aminotransferase, lactate dehydrogenase, and serum ferritin.[25]

Accumulating evidence suggests that the diagnosis of PH in patients with SCD acts as an independent predictor of mortality.[14] One study based on an estimate of median survival time of about 6.8 years after the diagnosis of PH found that those those with pulmonary hypertension died at a younger age compared to those without.[25]

As for arrhythmias, a study in the United States concluded that paroxysmal atrial fibrillation was the most common arrhythmia among patients with SCD crises, followed by supraventricular tachycardia, long QT syndrome, atrial flutter, and ventricular fibrillation.[26] In addition, a study among inpatients with SCD in the United States showed that 3.4% of them had documented arrhythmias. Of SCD-related admissions, 60% were associated with arrhythmia, with an obvious and clear rise in prevalence in subsequent years that significantly impacted care as it was associated with higher mortality in SCD patients and increased duration of hospitalization.[27]

After reviewing the literature, there were no updated studies regarding the incidence of PH risk and arrhythmias in adult patients with SCD in Saudi Arabia. Thus, we aimed to analyze it and provide data for future research, retrospectively.

Subjects and Methods

This retrospective study was done using hospital medical records at King Abdulaziz University Hospital (KAUH), a major tertiary care center in Jeddah, Saudi Arabia. We obtained ethical approval from the Unit of Biomedical Ethics Research Committee of KAUH (reference number 565–21; approved on 28 November 2023). Informed consent was not

required and was waived by the ethical committee as this study was a retrospective non-interventional study, and all information was collected from the medical records in the hospital. The data were anonymized and maintained with confidentiality in the office of the corresponding author in compliance with the Declaration of Helsinki. This study included all patients with a diagnosis of sickle cell disease who were followed between 2009 and 2021. We excluded patients with sickle cell anemia trait, pediatric patients below 15 years, and those who had never undergone an echocardiogram or electrocardiogram (ECG). Initially, 838 patients were screened, and after the application of the exclusion criteria, 517 patients' medical records were reviewed. All included patients had their records examined by a consultant cardiologist to screen for arrhythmias and assess the risk for PH according to the tricuspid regurgitation velocity observed in their echo, as adapted from the European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines.[28] Thereafter the patients were categorized into three risk groups: high risk when peak tricuspid regurgitation velocity (TVR) is 2.9−3.4 with other PH signs on echo or when >3.4; moderate risk when peak TVR is ≤2.8 or not measurable in the presence of other echo PH signs or peak TVR is 2.9–3.4 with no other PH signs; and low risk when peak TRV is ≤2.8 or not measurable, with no other echo PH signs.

For the purpose of data collection, a pre-designed checklist was prepared to collect data about patients' demographics (age, gender), death, type of hemoglobinopathy, comorbidities (cardiovascular diseases, diabetes mellitus, thyroid disease, history of pulmonary embolism (PE) or deep vein thrombosis (DVT), chronic kidney disease (CKD), pre-existing cardiac conditions (this included ischemic heart disease, valvular heart disease or rheumatic heart disease, liver cirrhosis, and hypertension [HTN]), in addition to patients' echocardiography reports and ECGs.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) program, version 26. To test the relationship between variables, qualitative data was expressed as numbers and percentages, and the chi-squared test was used to test the relationship between variables. Quantitative data was expressed as mean and standard deviation (mean ± SD). Multivariate logistic regression analysis was done to assess the association of risk factors with developing arrhythmias among studied patients. In addition, a multivariate ordered logistic regression model assessing the association of risk factors and risk for pulmonary hypertension was done. The odds ratio was calculated, and 95% confidence intervals (CIs) were reported. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 517 patients diagnosed with SCD were reviewed. Of these patients, 257 (49.7%) were female and 260 (50.3%) were male. Mean age was 34.45 ± 9.28 years. Upon investigation of different comorbidities, pre-existing cardiac conditions were the most prevalent (10.6%). Moreover, 7 (1.4%) had an arrhythmic episode, and 19 (3.7%) had a moderate risk of pulmonary hypertension and 17 (3.3%) were considered high risk. Of the included sample, 46 (8.9%) patients were deceased, with a mean age at death of 33 ± 10.3. Among those patients who had an arrhythmic episode, 2 developed atrial fibrillation, 3 developed first-degree heart block, 1 had supra-ventricular tachycardia, and 1 had a pulseless electrical activity. Demographic details and percentage distributions among studied patients are shown in Table 1. Based on echocardiography assessment, 11 (4.2%) and 10 (3.8%) males were found to have a high and moderate risk of PH, respectively, compared to 6 (2.3%) females with a high risk of PH and 9 (3.5%) with a moderate risk; there were no significant differences according to sex (p=0.467). Investigating the effect of the copresence of other comorbidities, PH showed a significant association with the following pre-existing cardiac conditions: venous thromboembolism, liver and kidney disease, and hypertension (p<0.000). In addition, among deceased patients, 7 (15.2%) had a high risk of PH while 2 (4.3%) had a moderate risk (p<0.000). Details of PH and its relationship to other variables are provided in Table 2. As for arrhythmias, most patients were male (5, 71.4%), in comparison to 2 females (28.6%), with no significant difference (p=0.26). There was a significant association between the presence of cardiac conditions and the development of arrhythmia, with 4 patients (57.1%) having pre-existing cardiac conditions (p<0.004). However, there was no significant association between having a history of PE and DVT with arrhythmias and not having such a history (1 patient [14.3%] and 6 patients [85.7%], respectively; p=0.259). Among patients with chronic kidney disease, two had a recorded incidence of arrhythmias, with a significant difference (p<0.001). Finally, there was a significant association between having PH and developing arrhythmias; two (28.6%) patients with arrhythmias were found to have a high risk of PH while three (42.9%) had a moderate risk (p<0.000) (Table 3).

Table 1 Distribution of Studied Patients According to Their Demographics, Chronic Diseases, PH Risks and Outcomes (N=517)

Variable	Number (%)
Demographics	
Age in years (mean ± SD)	35.5 ± 9.27
Male	260 (50.3)
Medical history	
Pre-existing cardiac condition	59 (11.4)
Thyroid disease	7 (1.4)
History of venous thromboembolism	26 (5)
Liver cirrhosis	5 (1)
Chronic kidney disease	6 (1.2)
Diabetes mellitus	7 (1.4)
Hypertension	36 (7)
RVSP (mean ± SD)	38.5± 17.36
Risk of pulmonary hypertension	
High risk	17 (3.3)
Moderate risk	19 (3.7)
Low risk	481(93)
Arrhythmia	7 (1.4)
Death	46 (8.9)

Table 2 Relationship Between Prevalence of Pulmonary Hypertension Risk and Patients' Demographics, Chronic Diseases, and Outcomes (N=517)

Variable	Pulmonary Hypertension Risk			p-value
	High number (%)	Moderate number (%)	Low number (%)	
Gender				
Female	6 (2.3)	9 (3.5)	242 (94.2)	0.467
Male	11 (4.2)	10 (3.8)	239 (91.9)	
Nationality				
Non-Saudi	11 (5)	5 (2.3)	206 (92.8)	0.067
Saudi	6 (2)	14 (4.7)	275 (93.2)	
Pre-existing cardiac condition				
Yes	11 (18.6)	8 (13.6)	40 (67.8)	<0.000
No	6 (1.3)	11 (2.4)	441 (96.3)	
Thyroid disease				
Yes	0 (0.0)	2 (28.6)	5 (71.4)	0.043
No	17 (3.3)	17 (3.3)	467 (93.3)	
Venous thromboembolism				
Yes	5 (19.2)	5 (19.2)	16 (61.5)	<0.000
No	12 (2.4)	14 (2.9)	465 (94.7)	

(*Continued*)

Table 2 (Continued).

Variable	Pulmonary Hypertension Risk			p-value
	High number (%)	Moderate number (%)	Low number (%)	
Liver cirrhosis Yes No	 1 (20) 16 (3.1)	 4 (80) 15 (2.9)	 0 (0) 481 (93.9)	 <0.000
Chronic kidney disease Yes No	 4 (66.7) 13 (2.5)	 2 (33.3) 17 (3.3)	 0 (0) 481 (94.1)	 <0.000
Diabetes mellitus Yes No	 0 (0) 17 (3.3)	 1 (14.3) 18 (3.5)	 6 (85.7) 475 (93.1)	 0.398
Hypertention Yes No	 6 (16.7) 11 (2.3)	 4 (11.1) 15 (3.1)	 26 (72.2) 455 (94.6)	 <0.000
Death Yes No	 7 (15.2) 10 (2.1)	 2 (4.3) 17 (3.6)	 37 (80.4) 444 (94.3)	 <0.000
Arrhythmia Yes No	 2 (28.6) 15 (2.9)	 3 (42.9) 16 (3.1)	 2 (28.6) 479 (93.9)	 <0.000

Logistic regression analysis was done to evaluate for independent risk factors and multiple significant results were noted (Tables 4 and 5). CKD, liver cirrhosis, arrhythmias, history of PE or DVT, HTN, and pre-existing cardiac conditions were all found to be independent risk factors for pulmonary hypertension in patients with sickle cell disease. Furthermore, patients with CKD were found to have 26.4 times higher odds of developing pulmonary hypertension than their counterparts (95% CI: 2.76–253.19; p=0.005). Also, patients with arrhythmias had 8.31 times increased odds of

Table 3 Relationship Between Prevalence of Arrhythmias and Patients' Demographics, Chronic Diseases, and Outcomes (N=517)

Variable	Arrhythmias		$\chi 2$	p-value
	Yes No. (%)	No No. (%)		
Age	33 ± 11.37	34.47 ± 9.26	6*	0.548
Gender Female Male	 2 (28.6) 5 (71.4)	 255 (50) 255 (50)	 1.26	 0.26
Nationality Non-Saudi Saudi	 2 (28.6) 5 (71.4)	 220 (43.1) 290 (56.9)	 0.59	 0.439
Pre-existing cardiac condition Yes No	 4 (57.1) 3 (42.9)	 55 (10.8) 455 (89.2)	 16.14	 0.004

(*Continued*)

Table 3 (Continued).

Variable	Arrhythmias		χ2	p-value
	Yes No. (%)	No No. (%)		
Thyroid disease Yes No	 0 (0.0) 7 (100)	 7 (1.4) 503 (98.6)	0.09	0.755
Venous thromboembolism Yes No	 1 (14.3) 6 (85.7)	 25 (4.9) 485 (95.1)	1.27	0.259
Liver cirrhosis Yes No	 0 (0.0) 7 (100)	 5 (1) 505 (99)	0.06	0.792
Chronic kidney disease Yes No	 2 (28.6) 5 (71.4)	 4 (0.8) 506 (99.2)	46.48	<0.001
Dabetes mellitus Yes No	 0 (0.0) 7 (100)	 7 (1.4) 503 (98.6)	0.09	0.755
Hypertension Yes No	 0 (0.0) 7 (100)	 36 (7.1) 474 (92.9)	0.53	0.466
Death Yes No	 2 (28.6) 5 (71.4)	 44 (8.6) 466 (91.4)	3.38	0.066

Table 4 Multivariate Ordered Logistic Regression for Pulmonary Hypertension

Variable	Odds ratio	p>|z|	[95% confidence interval]
CKD	26.42451	0.005	2.757854–253.1877
Liver cirrhosis	8.906725	0.022	1.368189–57.98159
Arrhythmias	8.31469	0.026	1.288201–53.66714
Venous thromboembolism	7.458677	0.000	2.646451–21.02131
Thyroid disease	4.737422	0.146	0.5810386–38.62595
Hypertention	3.549194	0.019	1.234815–10.20135
Pre-existing cardiac condition	3.166149	0.020	1.200802–8.348167

Table 5 Multivariate Logistic Regression for Arrhythmias

Variable	Odds ratio	p>|z|	[95% confidence interval]
Arrythmias	15.36027	0.013	1.770316–133.2745
CKD	6.28068	0.039	1.09699–35.95924
Pre-existing cardiac condition	0.0167641	0.031	0.0004059 – 0.6923091

pulmonary hypertension (95% CI: 1.29–53.67; p=0.026). On the other hand, thyroid disease was found to have no significant difference regarding pulmonary hypertension risk (p=0.146).

Additionally, patients with CKD were found to have 15.36 times higher odds of developing cardiac arrhythmias in comparison to patients without CKD (95% CI: 1.77–133.27; p=0.013); however, patients with pre-existing cardiac conditions were found to have 6.28 times higher odds than patients without them (95% CI: 1.09–35.96; p=0.039).

Discussion

Patients with sickle cell disease are at high risk for many complications, including cardiac and pulmonary complications. In this study we retrospectively reviewed SCD patients at a tertiary care center in a region with a high prevalence of the disease. Sickle cell disease patients who had undergone echocardiographic evaluation and had had an ECG assessment were analyzed. The study found that around 3.3% of patient had a high risk of PH and 3.7% had a moderate risk based on tricuspid regurgitation velocity observed in their echo, as adapted from ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension.[28] Patients with other comorbidities, including CKD, liver cirrhosis, arrhythmias, VTE, HTN, and pre-existing cardiac conditions, were found to have higher odds of being at risk of PH. Only 1.4% of the patients assessed had arrythmia; a history of CKD and pre-existing cardiac conditions carried the highest odds.

Though echocardiographic assessment can suggest a diagnosis of PH and establish a risk category based on peak TVR velocity, a right heart catheterization is usually required to confirm diagnosis as it is the gold standard test.[29] Studies that look at patients with SCD who have undergone right heart catheterization are likely under-reporting the prevalence of this important complication in this vulnerable patient population; the invasive nature of this procedure and the cost and technical difficulties associated with it do not allow all patients to undergo such a workup. Therefore, in this study we preferred to evaluate the risk of PH by echocardiography to capture all symptomatic and asymptomatic SCD patients.

The significant association between VTE and risk of PH reported in this study (p<0.000) is in keeping with a similar study which explained how sickle cell disease by itself is associated with chronic hypercoagulable states via various mechanisms, including the chronic hemolysis that increases the risk of VTE but also contributes to the pathogenesis behind developing PH. The researchers also found an increased risk of VTE by 30% or more depending on the SCD genotype in patients with pulmonary hypertension. The occurrence of thromboembolic events in the pulmonary blood vessels is underestimated and sometimes misdiagnosed in SCD patients, as some of them will have no signs or symptoms of acute venous thromboembolism or pulmonary embolism. However, when imaging by CT scan or ventilation perfusion scan of the lung is performed for any reason, an incidental finding of a thromboembolic event or its consequences is usually found. This was supported in a study that demonstrated the presence of acute thromboembolic events in more than 50% of SCD patients, and a ventilation perfusion scan of the lung revealed mismatched segmental perfusion defects in around 80% of cases.[30] This finding signifies the need for further preventative measures to ensure reduction of VTE risk in this patient group.[31] In addition, this study shows what several studies have documented before regarding the significant relation between pre-existing cardiac conditions, pulmonary hypertension, and SCD.[32,33] Furthermore, pulmonary hypertension risk was associated with chronic kidney disease (p<0.000); all six patients had a moderate to high risk (33.3% and 66.7%, respectively). This finding is consistent with another study that postulated common mechanisms shared in the pathogenesis of CKD that could also exacerbate or induce PH, such as volume overload, severe anemia, and left ventricular dysfunction. However, this study was not specific to patients with sickle cell disease.[34] Moreover, the study commented that correcting volume overload and treatment of left ventricular disorders can help reduce PH in patients with CKD. The statistically significant association between pulmonary hypertension and increased mortality in patients with SCD shown in this study has been documented in previous prospective and registry-based studies.[35,36] This finding calls for more attention to implementing strategies to enhance early diagnosis of PH and trials to assess treatment options and to establish guidelines for the follow-up and management of SCD patients.

The anemia and polymerization of hemoglobin can lead to cardiac involvement in SCD, whether it is left or right ventricular dysfunction.[37] In this study, we investigated the presence of arrhythmias in patients with SCD. Only seven patients were found to have arrhythmias, while in a similar study, which included 100 patients with SCD, 41% were found to have arrhythmias, mainly sinus tachycardia.[38] The discrepancy of our finding compared to the other aforementioned study could be due to the timing of ECG assessments, which were conducted outside the occurrence of arrhythmia, and potentially influenced the observed results. Additionally, 5 out of 7 patients with arrhythmias were found to have a moderate to high risk of PH (p<0.000). Similarly, the

relationship between PH and arrhythmias has been reported in previous studies; one concluded that QTc dispersion is associated with SCD, especially in those patients with PH.[39] Moreover, about 50% of patients with CKD had arrhythmias with a significant difference ($p<0.000$). It was noted in a previous study that patients on hemodialysis have various arrhythmic triggers, including left ventricular dysfunction and fluid overload.[34] Despite the low incidence of arrhythmias found in SCD patients, most of the events were observed in the sub-groups who have CKD or a high risk of developing PH, suggesting that screening for arrhythmias is recommended in those sub-groups. In addition, hemodialysis is associated with significant alterations in electrolyte levels, with gradual shifts occurring between dialysis sessions and rapid changes during the dialysis procedure. The established risk of arrhythmias related to these electrolyte fluctuations, along with the chronic and often severe electrolyte imbalances prevalent in individuals with CKD, is widely recognized.[40] Therefore, implementing cardiovascular risk modification and prevention of electrolyte disturbances can be beneficial.[40] In this study, arrhythmias were found to have no significant association with death as an outcome in patient condition ($p=0.066$), contrary to other studies in the literature that mention them as a risk factor for increased mortality and length of stay.[38] Disparity in this result could be due to the small number of documented arrhythmic patients in our data, or maybe the presence of other comorbidities associated with arrhythmia in SCD patients in the other literature described here contributed to the outcome directly or indirectly.

The strength of this study mainly comes from representing real-world data in a country with a high prevalence of sickle cell disease. On the other side, this study has many limitations, mainly its retrospective nature and the challenges related to patients' loss of follow-up, which may introduce gaps in patient data. Another limitation is that the documented cases of arrhythmia were small and we could not be certain in predicting their morbidity and mortality.

In conclusion, adult patients with SCD are at risk of developing pulmonary hypertension and various arrhythmias. However, this risk is often underestimated and underdiagnosed in this population despite their great significance and effect on patients' survival. This study highlights the importance of strategies that can potentially serve as a preventative approach, such as: addressing fluid and electrolyte imbalances, implementing interventions to lower cardiovascular risk, and involving a multidisciplinary healthcare team in the care of adult SCD patients, even if they are asymptomatic. Moreover, further research is needed regarding the relationship between chronic kidney disease and pulmonary hypertension in the setting of sickle cell disease, which can help to improve our understanding and hopefully achieve better care of such patients. Also, studies using a prospective design are recommended for better identification of pulmonary hypertension and arrhythmias in patients with SCD to establish screening programs for early prediction and decreased mortality.

Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Can COVID-19 Increase Platelet in Adult Immune Thrombocytopenia During the TPO-RA Administration? A Real-World Observational Study

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Introduction: COVID-19 infection has brought new challenges to the treatment of adult patients with immune thrombocytopenia (ITP). In adult ITP patients, there have been no relevant reports exploring the incidence, clinical characteristics, and risk factors of platelet elevation after COVID-19 infection.

Materials and Methods: A total of 66 patients with previously diagnosed ITP from December 2022 to February 2023 in a single-center were collected and analyzed for this real-world clinical retrospective observational study.

Results: In the platelet count increased group (n = 19), 13 patients (68.4%) were using thrombopoietin receptor agonists (TPO-RA) treatment at the time of COVID-19 infection; the median platelet count was 52 (2–207) $\times 10^9$/L at the last visit before infection and 108 (19–453) $\times 10^9$/L at the first visit after infection. In the platelet count stable group (n = 19) and platelet count decreased group (n = 28), 9 (47.4%) and 8 (28.6%) patients were using TPO-RA at the time of infection, respectively. ITP patients treated with TPO-RA had a significantly higher risk of increased platelet count than those not treated with TPO-RA at the time of infection (platelet count increased group vs platelet count decreased group: OR: 5.745, p = 0.009; platelet count increased group vs the non-increased group: OR: 3.616, p = 0.031). In the platelet count increased group, the median platelet count at 6 months post-infection was 67 (14–235) $\times 10^9$/L, which was significantly higher than the platelet level at the last visit before infection (p = 0.040).

Conclusion: This study showed that some adult ITP patients had an increase in platelet count after COVID-19 infection, and this phenomenon was strongly associated with the use of TPO-RA at the time of infection. Although no thrombotic events were observed in this study, it reminds clinicians that they should be alert to the possibility of thrombotic events in the long-term management of adult ITP patients during the COVID-19 pandemic.

Keywords: immune thrombocytopenia, thrombopoietin receptor agonists, COVID-19, thrombosis

Introduction

Since the novel coronavirus (COVID-19) outbreak in December 2019, the COVID-19 pandemic caused by SARS-CoV-2 virus has brought new challenges to the management of adult patients with immune thrombocytopenia (ITP). The occurrence and recurrence of ITP is closely associated with viral infections such as cytomegalovirus, hepatitis C virus (HCV) and Epstein-Barr virus (EBV).[1] Antibodies induced by viral infections may cross-react with platelets thereby leading to platelet destruction.[2] COVID-19 infection has been identified as a risk factor for the development of ITP,[3] and the occurrence and relapse of ITP induced by COVID-19 infection has been frequently reported.[4–7] This may be related to the direct invasion of hematopoietic stem cells in the bone marrow by COVID-19, autoimmune destruction of platelets

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and increased platelet depletion due to the formation of microthrombi.[8,9] Corticosteroids and high-dose intravenous immunoglobulin (IVIG) are the most commonly used regimens for the treatment of COVID-19 associated thrombocytopenia.

However, two small-sample cases studies found that patients with chronic ITP (cITP) develop early thrombocytosis after COVID-19 infection, which is common in cITP treated with thrombopoietin receptor agonists (TPO-RA).[10,11] A single-center observational cohort study conducted at Beijing Children's Hospital demonstrates a transient rise in platelet counts after COVID-19 infection in cITP children treated with TPO-RA.[12] However, in adult ITP patients, there have been no relevant reports exploring the incidence, clinical characteristics, and risk factors of platelet elevation after COVID-19 infection. To further verify and explore the impact of COVID-19 infection in adult ITP patients, this study retrospectively analyzed the clinical data of adult patients with ITP in our center from December 2022 to February 2023 to provide a reference for the management of adult ITP patients during the COVID-19 pandemic.

Materials and Methods

Data Collection

A total of 66 patients with previously diagnosed ITP in the First Affiliated Hospital of University of Science and Technology of China (Anhui Provincial Hospital) from December 2022 to February 2023 were collected for this real-world clinical retrospective observational study. Patient's selection criteria: (1) diagnosed as ITP; (2) infected COVID-19 during this period; (3) age above 18 years old.

The diagnosis and follow-up information of ITP patients were obtained from the electronic medical record system or telephone follow-up. The data collected included demographic data, ITP treatment modalities, platelet count at the last visit before COVID-19 infection and platelet count at the first visit after COVID-19 infection. Platelet counts at 3 months and 6 months post-COVID-19 infection were tracked for ITP patients in the platelet count increased group. This study protocol was approved by the medical research ethics committee of the First Affiliated Hospital of University of Science and Technology of China and was conducted in accordance with the Declaration of Helsinki (2023-RE-322). Informed consent was waived by the medical research ethics committee of the First Affiliated Hospital of University of Science and Technology of China because of retrospective nature of the study.

Classifications

According to the platelet counts of the patients at the first visit after COVID-19 infection, the patients were divided into platelet count increased group, platelet count stable group and platelet count decreased group. "Platelet count increased group" was defined as an increase of more than 20% in the platelet count at the first visit after COVID-19 infection compared with the last visit before infection. "Platelet count stable group" was defined as the platelet count fluctuating by less than 20% at the first visit after COVID-19 infection compared to the last visit before infection. "Platelet count decreased group" was defined as a decrease of more than 20% in the platelet count at the first visit after COVID-19 infection compared with the last visit before infection.

Definitions and Statistical Analysis

Newly diagnosed ITP (nITP) is defined for patients with ITP within 3 months of diagnosis; persistent ITP (pITP) is defined for patients with ITP lasting between 3 and 12 months from diagnosis; chronic ITP (cITP) is defined for patients with ITP lasting for more than 12 months.[13] First-line treatment refers to conventional treatment with glucocorticoids and/or intravenous immunoglobulin to improve platelet count; second-line treatment involved the use of one or more of the following three therapies: thrombopoietic agents, rituximab, or splenectomy; third-line treatment refers to the use of therapeutic regimens supported by prospective multicenter clinical trials including decitabine, all-trans retinoic acid (ATRA) in combination with danazol, and other drugs.[14]

Continuous variables were described as means and standard deviations or medians and ranges. Categorical variables were described as frequencies and proportions. Continuous variables were compared between groups by Kruskal–Wallis *H*-test. Test for association between categorical variables used by chi-square test or Fisher's exact test (if applicable). The

potential risk factors associated with fluctuation in platelet counts were further explored using binary logistic regression analysis. Increased, stable, or decreased platelet counts were used as dependent variables, and age, gender, whether corticosteroids were being used at the time of infection, and whether TPO-RA was being used at the time of infection as the independent variables. SPSS26.0 software was used for statistical analysis and differences were considered statistically significant at $p< 0.05$.

Results

Clinical Characteristics

A total of 66 adult ITP patients with COVID-19 infection were enrolled, including 25 (37.9%) males and 41 (62.1%) females, with an average age of 48±16 years. Among them, 10 patients (15.1%) had other autoimmune diseases (6 patients with undifferentiated connective tissue disease, 2 patients with hypothyroidism, 1 patient with Sjogren's syndrome and 1 patient with Evans syndrome), and 3 patients (4.5%) underwent previous splenectomy. There were 11 patients (16.7%) with nITP, 16 patients (24.2%) with pITP, and 39 patients (59.1%) with cITP. Before COVID-19 infection, 18 patients (27.3%) of these patients had received no prior treatment, 10 patients (15.1%) had received first-line treatment, 37 patients (56.1%) had received second-line treatment, and 1 patient (1.5%) had received third-line treatment (Table 1).

Table 1 Clinical Characteristics

Characteristics	All Patients (n=66)
Age [years, Mean±SD]	48±16
Gender [n (%)]	
Male	25 (37.9)
Female	41 (62.1)
ITP status [n (%)]	
Newly diagnosed ITP	11 (16.7)
Persistent ITP	16 (24.2)
Chronic ITP	39 (59.1)
History of autoimmune disease other than ITP [n (%)]	
Undifferentiated connective tissue disease	6 (9.1)
Hypothyroidism	2 (3.0)
Sjogren syndrome	1 (1.5)
Evans syndrome	1 (1.5)
Splenectomy [n (%)]	3 (4.5)
ITP treatment lines before COVID-19 [n (%)]	
Untreated	18 (27.3)
1	10 (15.1)
2	37 (56.1)
3	1 (1.5)
Treatment at the last visit before infection [n (%)]	
No current treatment	23 (34.8)
Corticosteroid only	11 (16.7)
TPO-RA only	22 (33.3)
TPO-RA + corticosteroid	3 (4.5)
Other drug combinations*	7 (10.6)
Platelet count at the last visit before infection [$\times 10^9$/L, Median (range)]	64 (2–246)
Time from last visit before infection to infection [days, Median (range)]	16 (1–110)
Platelet count at the first visit after infection [$\times 10^9$/L, Median (range)]	40 (2–453)
Time from infection to first visit after infection [days, Median (range)]	16 (1–82)

Abbreviations: ITP, immune thrombocytopenia; TPO-RA, thrombopoietin receptor agonists. *Other drug combinations including Eltrombopag plus Cyclosporine (n=1), Eltrombopag plus Danazol (n=1), Hetrombopag plus Cyclosporine (n=1), Hetrombopag plus Corticosteroid plus Cyclosporine (n=1), Hetrombopag plus Corticosteroid plus Rituximab (n=1), Corticosteroid plus Azathioprine plus Danazol (n=1), Decitabine plus Chidamide (n=1).

At the time of COVID-19 infection, 11 patients (16.7%) received corticosteroids monotherapy, 22 patients (33.3%) received TPO-RA regimens monotherapy, 3 patients (4.6%) received corticosteroids and TPO-RA combinations and 7 patients (10.6%) received other combinations treatment. Twenty-three patients (34.8%) did not receive any regimens. TPO-RA using details were shown in Supplementary Table 1.

COVID-19 Infection and Platelet Count Fluctuation

The median platelet count of 66 ITP patients at the last visit before COVID-19 infection was 64 (2–246) $\times 10^9$/L, and the median time from the last visit before infection to COVID-19 infection was 16 (1–110) days. The median platelet count at the first visit after infection was 40 (2–453) $\times 10^9$/L, and the median time from COVID-19 infection to the first visit was 16 (1–82) days (Table 1). After COVID-19 infection, the platelet count of 19 patients (28.8%) increased by more than 20% compared with that before infection (platelet count increased group), of which 14 patients (73.7%) increased by more than 50%. The platelet count of 19 patients (28.8%) was stable which fluctuated within 20% compared with that before infection (platelet count stable group). Twenty-eight patients (42.4%) had a reduction in platelet count of more than 20% (platelet count decreased group), of which 17 (60.7%) patients had a decrease of 50% or more (Figure 1).

There were 19 patients in the platelet count increased group, including 6 (31.6%) males and 13 (68.4%) females, with an average age of 51±14 years. Among these patients, 2 patients (10.5%) were nITP, 4 patients (21.1%) were pITP, and 13 patients (68.4%) were cITP. One patient (5.3%) had received first-line treatment, 13 patients (68.4%) had received second-line treatment, 1 patient (5.3%) had received third-line treatment, and four patients (21.1%) had received no previous treatment. At the time of COVID-19 infection, 4 patients (21.1%) were using corticosteroids and 13 patients (68.4%) were using TPO-RA. The median platelet count at the last visit before infection was 52 (2–207) $\times 10^9$/L, and the median platelet count at the first visit after infection was 108 (19–453)$\times 10^9$/L. The median time from the last visit before infection to COVID-19 infection was 13 (1–67) days, and the median time from infection to the first visit after infection was 20 (2–47) days (Table 2).

A total of 19 patients in the platelet count stable group include 8 (42.1%) males and 11 (57.9%) females, with an average age of 44±16 years. Of these patients, 9 (47.4%) were using TPO-RA at the time of infection. The median platelet count at the last visit before infection was 64 (14–246) $\times 10^9$/L, and the median platelet count at the first visit after infection was 64 (12–259) $\times 10^9$/L. The median time from the last visit before infection to COVID-19 infection was 20 (4–86) days, and the median time from infection to the first visit after infection was 21 (5–57) days (Table 2). There were 28 patients in the platelet count decreased group, including 11 (39.3%) males and 17 (60.7%) females, with a mean

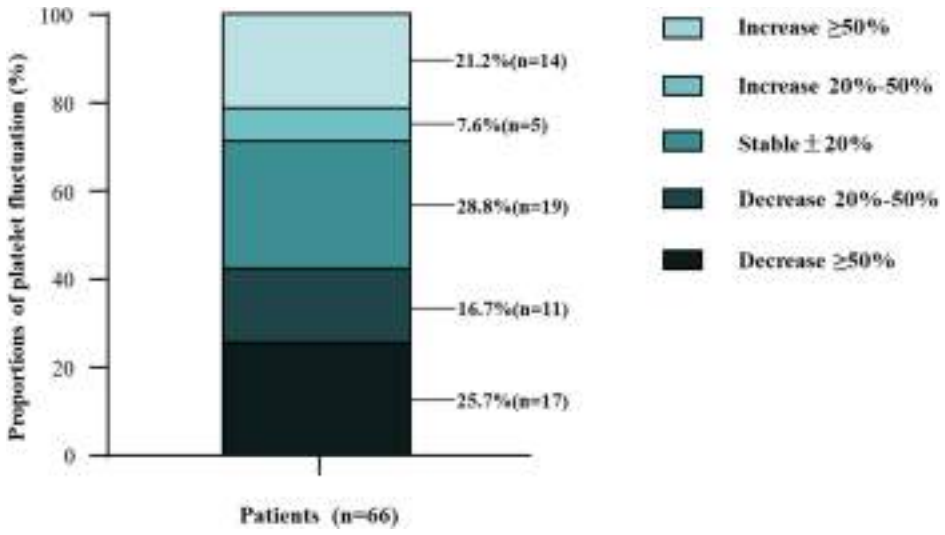

Figure 1 COVID-19 infection and platelet count fluctuation. After COVID-19 infection, the platelet count of 19 patients increased by more than 20% compared with that before infection, of which 14 patients increased by more than 50%. The platelet count of 19 patients was stable which fluctuated within 20% compared with that before infection. Twenty-eight patients had a reduction in platelet count of more than 20%, of which 17 patients had a decrease of 50% or more.

Table 2 Clinical Characteristics of the Increased, Stable and Decreased Platelet Count Groups

Characteristics	Increased Platelet Count (n=19)	Stable Platelet Count (n=19)	Decreased Platelet Count (n=28)	*P*-value
Age [years, Mean±SD]	51±14	44±16	48±18	0.427
Gender [n (%)]				0.783
Male	6 (31.6)	8 (42.1)	11 (39.3)	
Female	13 (68.4)	11 (57.9)	17 (60.7)	
ITP status [n (%)]				0.532
Newly diagnosed ITP	2 (10.5)	3 (15.8)	6 (21.4)	
Persistent ITP	4 (21.1)	3 (15.8)	9 (32.2)	
Chronic ITP	13 (68.4)	13 (68.4)	13 (46.4)	
ITP treatment lines before COVID-19 [n (%)]				0.079
Untreated	4 (21.1)	5 (26.3)	9 (32.1)	
1	1 (5.3)	1 (5.3)	8 (28.6)	
2	13 (68.4)	13 (68.4)	11 (39.3)	
3	1 (5.3)	0	0	
Treated with corticosteroid at the last visit before infection [n (%)]	4 (21.1)	5 (26.3)	8 (28.6)	0.936
Treated with TPO-RA at the last visit before infection [n (%)]	13 (68.4)	9 (47.4)	8 (28.6)	0.020
Platelet count at the last visit before infection [$\times10^9$/L, Median (range)]	52 (2–207)	64 (14–246)	71 (14–227)	0.182
Time from last visit before infection to infection [days, Median (range)]	13 (1–67)	20 (4–86)	14 (2–110)	0.381
Platelet count at the first visit after infection [$\times10^9$/L, Median (range)]	108 (19–453)	64 (12–259)	23 (2–90)	<0.001
Time from infection to first visit after infection [days, Median (range)]	20 (2–47)	21 (5–57)	13 (1–82)	0.104

Abbreviations: ITP, immune thrombocytopenia; TPO-RA, thrombopoietin receptor agonists.

age of 48 ± 18 years. Among them, 8 patients (28.6%) were receiving TPO-RA before infection. The median platelet count at the last visit before infection was 71 (14–227) $\times10^9$/L, and the median platelet count at the first visit after infection was 23 (2–90) $\times10^9$/L. The median time from the last visit before infection to COVID-19 infection was 14 (2–110) days, and the median time from infection to the first visit after infection was 13 (1–82) days (Table 2).

Risk Assessment of Platelet Count Elevation After COVID-19 Infection

There were no significant differences in age, gender, ITP stage, previous treatment lines, use of corticosteroids at the time of infection, platelet count at the last visit before infection, the time from the last visit before infection to COVID-19 infection, and the time from COVID-19 infection to the first visit after infection among the ITP patients with above three groups. However, there was a significant difference in whether TPO-RA was being used at the time of infection (p=0.020) (Table 2).

ITP patients treated with TPO-RA had a significantly higher risk of increased platelet count than those not treated with TPO-RA at the time of infection [platelet count increased group vs platelet count decreased group: OR (95% CI): 5.745 (1.556–21.216), p=0.009; platelet count increased group vs the non-increased group (stable and decreased groups): OR (95% CI): 3.616 (1.123–11.648), p=0.031] (Figure 2).

Dynamics of Platelet Levels in Patients in the Platelet Count Increased Group

In the platelet count increased group, the median platelet count at the first visit after COVID-19 infection was 108 (19–453)$\times10^9$/L, which was significantly higher than that at the last visit before infection [52 (2–207)$\times10^9$/L] (p<0.001) (Figure 3A). The median platelet count at 3 months post-infection was 58 (9–240) $\times10^9$/L, which was slightly higher than that at the last visit before infection (p=0.180)(Figure 3B). The median platelet count at 6 months post-infection was 67 (14–235) $\times$ 10^9/L, which was significantly higher than the platelet level at the last visit before infection (p=0.040) (Figure 3C). Although platelet counts at 3 months and 6 months after infection were significantly lower than those at the first visit after infection (p=0.011, 0.038) (Figure 3D); however, there was no difference between platelet counts at 3 months and 6 months (p=0.561) (Figure 3D).

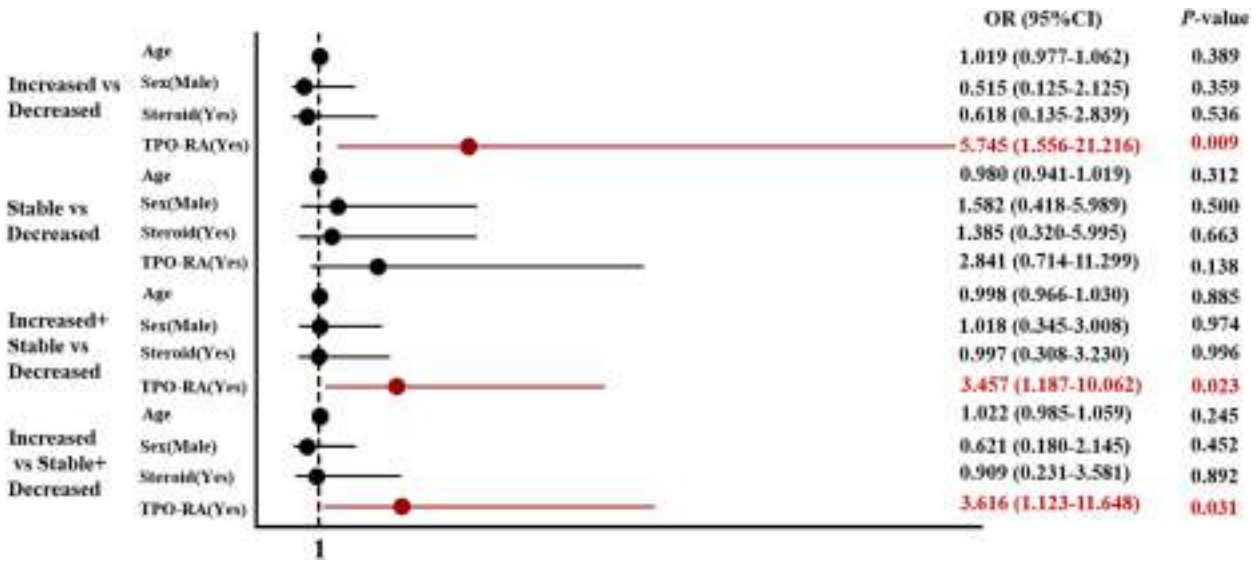

Figure 2 Risk factors of ITP patients with increased platelet count after COVID-19 infection.

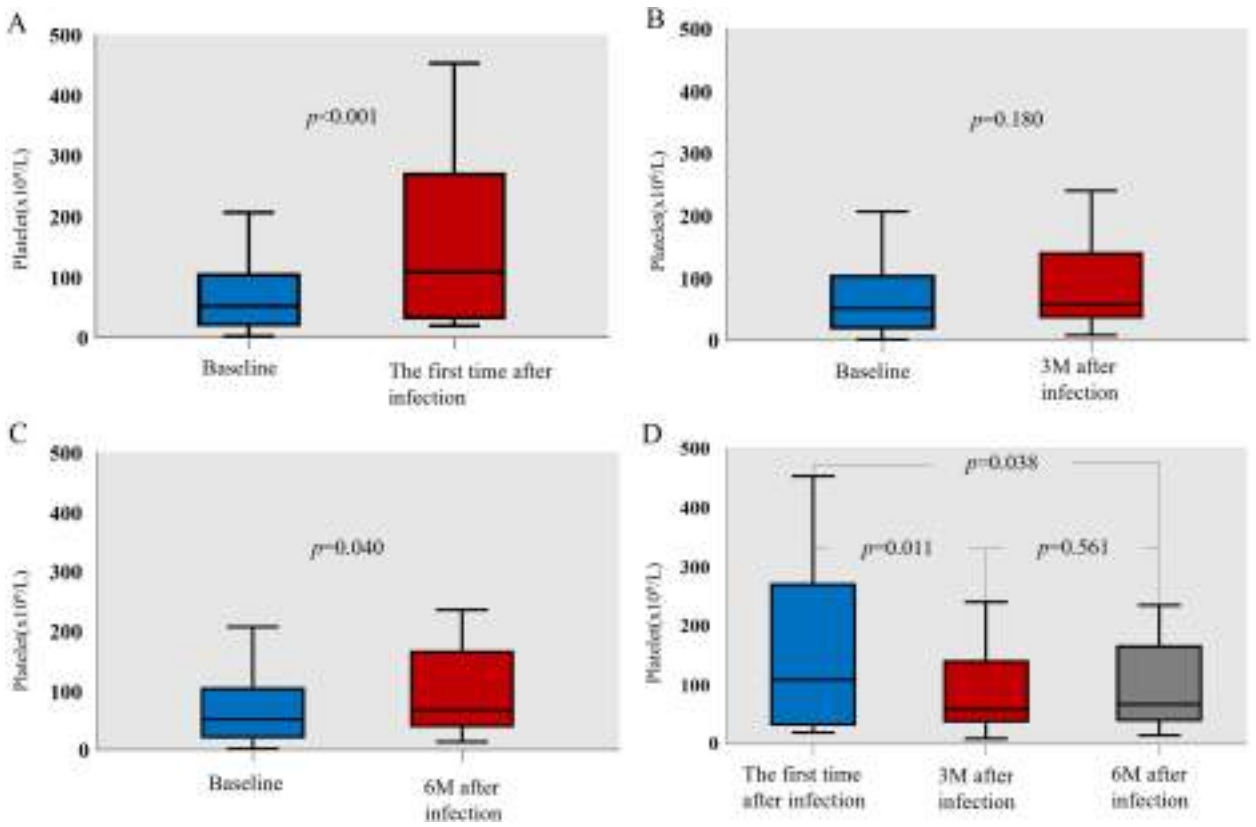

Figure 3 Changes of platelet counts in patients of platelet count increased group. Platelet counts at the first visit after COVID-19 infection compared with the last visit before infection (**A**). Platelet counts at the 3 months after COVID-19 infection compared with the last visit before infection (**B**). Platelet counts at the 6 months after COVID-19 infection compared with the last visit before infection (**C**). Platelet counts at the first visit, at the 3 months and 6 months after COVID-19 infection which compared with each other (**D**).

Discussion

In this study, 28.8% of adult ITP patients had an increase in platelet count after COVID-19 infection (more than 20% above baseline). Logistic regression analysis showed that platelet count elevation was strongly associated with the use of TPO-RA. This is consistent with two previous small-sample cases studies,[10,11] and a study of pediatric ITP patients that reported a transient increase in platelet counts after COVID-19 infection in cITP patients treated with TPO-RA;[12] the peak rise in platelet counts occurred 1–2 weeks after COVID-19 infection, and platelet counts returned to pre-infection levels 3–4 weeks after infection.[10–12] However, this study indicated that, in the platelet count increased group, platelet counts elevation mostly occurred within 1 month after COVID-19 infection; the platelet count was slightly increased at 3 months after infection and the platelet count was still at a higher level at 6 months after infection when compared with that before infection (p=0.180, 0.040).

TPO-RA use synergized with cytokines and lymphopenia caused by COVID-19 infection might contribute to the elevation of platelet count in ITP patients. After COVID-19 infection, there was a significant increase in the levels of many inflammatory cytokines, such as IL-6, IL-11 and tumor necrosis factor (TNF-α).[15] Related studies have shown that these cytokines could promote megakaryocyte production,[16] and Stone et al suggested that IL-6 promotes megakaryopoiesis by stimulating hepatic TPO expression.[17] In addition, SARS-CoV-2 virus can directly infect and induce apoptosis of lymphocytes and relevant studies have shown a decrease in peripheral blood lymphocytes in COVID-19-infected patients, especially CD4+ and CD8+ T lymphocytes,[18] which leads to a decrease in platelet antibody production and further reduces platelet destruction. In the future, we will further expand the sample size and further analyse whether the duration, dose and frequency of TPO-RA use affects the extent of platelet increase.

This study suggested that COVID-19 infection in ITP patients treated with TPO-RA may lead to a further increase in platelet counts. Although no thrombotic events were observed in this study, it reminds clinicians that they should be paid close attention to the possibility of thrombotic events in the long-term management of ITP. Firstly, although ITP is an acquired autoimmune hemorrhagic disease, ITP itself is also a high-risk factor for thrombosis;[19] a meta-analysis of three large population-based observational studies which conducted in Denmark, the United Kingdom and the United States indicated that the annual incidence of arterial and venous thrombotic events in patients with ITP ranged from 1.0 to 2.8 per 100 and 0.4 to 0.7 per 100, respectively, which were significantly higher than that in non-ITP patients (0.7 to 1.8 per 100 patients and 0.1 to 0.4 per 100 patients, respectively).[20] Secondly, pro-inflammatory cytokines release, platelet adhesion and aggregation, endothelial inflammation and injury, thrombin generation caused by SARS-CoV-2 virus infection might lead to immunothrombosis.[21,22] Epidemiologic studies indicated a high incidence of venous thromboembolic events (VTEs), including deep vein thrombosis (DVT) and pulmonary embolism (PE), in patients with COVID-19;[23–25] COVID-19 might also be associated with an increased incidence of arterial thrombotic events such as ischemic stroke and limb ischemia, especially in patients with severe COVID-19.[26–28] Thirdly, TPO-RA increases platelet production by binding to and activating the thrombopoietin receptor on the membrane of megakaryocytes.[29] Studies have shown that the incidence of arterial and venous thrombotic events in cITP patients treated with TPO-RA is 2 to 3 times higher than that in patients who do not receive TPO-RA,[30,31] which may be related to megakaryocyte activation and platelet production rapid elevation.

Conclusion

In conclusion, this study showed that some adult ITP patients had an increase in platelet count after COVID-19 infection, and this phenomenon was strongly associated with the use of TPO-RA at the time of infection. However, there are some limitations in this study. First, this was a retrospective study with a small sample size and the follow-up time was relatively short. In addition, there is no clear and uniform time point which designed for observing the platelet levels fluctuation after COVID-19 infection due to the essence of retrospective research, resulting in the possibility that the recording of platelet counts after infection may be somewhat biased from the actual situation. Finally, this study did not focus on the changes of coagulation function, including D-dimer levels, in patients with elevated platelet counts during the follow-up period; therefore, there is insufficient data to prove the possibility of thrombotic events in this group patients.

Abbreviations

ITP, Thrombocytopenia; TPO-RA, Thrombopoietin receptor agonists; COVID-19, Novel coronavirus; cITP, Chronic ITP; nITP, Newly diagnosed ITP; pITP, Persistent ITP; ATRA, All-trans retinoic acid; TNF-α, Tumor necrosis factor; VTEs, Venous thromboembolic events; DVT, Deep vein thrombosis; PE, Pulmonary embolism.

Data Sharing Statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics and Consent to Participate

This study was approved by the ethics committees of the First Affiliated Hospital of University of Science and Technology of China and was conducted in accordance with the Declaration of Helsinki (2023-RE-322). Informed consent was waived by the medical research ethics committee of the First Affiliated Hospital of University of Science and Technology of China because of retrospective nature of the study. All data were secured, protected, and accessed only for the authors. Personal information relating to the data that identifies the research subject is replaced with a numerical number. The descriptive data were summarized by tables and figures.

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.

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REVIEW

Eosinophils and Cognitive Impairment in Schizophrenia: A New Perspective

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Abstract: Schizophrenia is a complex psychiatric disorder characterized by a wide array of cognitive impairments. While research has predominantly focused on the neurological aspects of schizophrenia, emerging evidence suggests that the immune system, specifically eosinophils, may play a significant role in the cognitive deficits associated with the disorder. This review presents a novel perspective on the interplay between eosinophils and cognitive impairment in schizophrenia. Eosinophils, traditionally associated with allergic responses and inflammation, have garnered limited attention within the realm of neuropsychiatry. Recent studies have hinted at a potential link between eosinophil activation and the pathogenesis of schizophrenia. In this comprehensive review, we delve into the world of eosinophils, elucidating their nature, functions, and interactions with the immune system. We examine the cognitive deficits observed in individuals with schizophrenia and discuss existing theories on the etiology of these impairments, focusing on immune system involvement. The paper also highlights the evolving body of research that supports the idea of eosinophilic influence on schizophrenia-related cognitive deficits. Furthermore, we explore potential mechanisms through which eosinophils may exert their effects on cognitive function in schizophrenia, including interactions with other immune cells and inflammatory pathways. By discussing the clinical implications and potential therapeutic avenues stemming from this newfound perspective, we underscore the practical significance of this emerging field of research. While this paper acknowledges the limitations and challenges inherent in studying eosinophils within the context of schizophrenia, it serves as a posit for novel thought in this vexing disease space as well as a call to action for future research endeavors. By providing a comprehensive survey of the existing literature and posing unanswered questions, we aim to inspire a reimagining of the relationship between eosinophils and cognitive impairment in schizophrenia, ultimately advancing our understanding and treatment of this debilitating disorder.

Keywords: eosinophils, cognitive impairment, schizophrenia

Introduction

Schizophrenia, a severe and enigmatic neuropsychiatric disorder, has long captured the interest of researchers, clinicians, and society as a whole.[1] Formerly designated as dementia praecox and characterized by a complex interplay of symptoms that include hallucinations, delusions, disorganized thinking, and emotional dysregulation, schizophrenia extends its impact far beyond the boundaries of the mind.[2] One aspect of this disorder that has gained increasing attention in recent years is the cognitive impairment that frequently accompanies it.[3] Cognitive deficits in schizophrenia significantly hinder individuals' daily functioning, quality of life, and long-term outcomes.[4] Historically, research into the cognitive aspects of schizophrenia has predominantly traversed the neurological terrain, examining factors including neurotransmitter imbalances and brain structure abnormalities.[5] Yet, as we delve deeper into the intricacies of this complex disorder, an emerging perspective suggests that the immune system, often left in the shadows, may hold a key to understanding some of schizophrenia's cognitive mysteries.

This paper presents a novel perspective on the potential role of eosinophils, a subtype of white blood cells primarily known for their involvement in allergic responses and inflammation, in cognitive impairment associated with schizophrenia. While eosinophils have not been a central focus in the realm of neuropsychiatry, recent studies and evolving

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hypotheses suggest that they may play a significant part in the pathogenesis and progression of schizophrenia.[6] The immune system's connection to mental health has been a burgeoning field of study, with mounting evidence pointing to immune dysregulation as a contributing factor in various neuropsychiatric disorders.[7] In schizophrenia, the notion of immune involvement has gradually shifted from the periphery to the center of attention, and eosinophils, with their multifaceted roles within the immune system, have been drawn into the spotlight.[8]

The objectives, herein, are to elucidate the nature and functions of eosinophils, to delve into the cognitive deficits that define schizophrenia, and to critically examine the evidence and hypotheses surrounding eosinophilic involvement in this disorder. Furthermore, we will explore potential mechanisms through which eosinophils might influence cognitive function in schizophrenia, as well as the clinical implications and therapeutic potential that this new perspective may offer. In this exploration, it becomes increasingly clear that schizophrenia is a disorder with a web of influences, and the role of eosinophils in cognitive impairment represents a new thread that warrants careful examination. By providing a posit for novel thought in this vexing disease space as well as integrating this emerging perspective into the broader context of schizophrenia research, we hope to contribute to a deeper understanding of the disorder and, in doing so, open doors to novel insights and potential interventions.

Methods

The rationale for investigating the relationship between eosinophils and cognitive impairment in schizophrenia is listed in Table 1. It includes a multifaceted approach for this new perspective and rooted in both clinical observations and emerging scientific evidence. The quality of included studies was assessed using appropriate tools such as the Newcastle-Ottawa Scale for observational studies or the Cochrane Risk of Bias tool for randomized controlled trials.

Table 1 Selection Criteria and Approach for Publication Selection. Search Words Employed Included Medical Subject Headings (MeSH), Terms and Keywords: "Eosinophils", "Schizophrenia", "Cognitive Impairment", "Neurocognitive Dysfunction", "Cognitive Deficits", "Psychosis", "Blood Eosinophil Count", "Peripheral Eosinophils", "Immune System", "Inflammation", "Neuroinflammation", "Cytokines", "Interleukins", "Tumor Necrosis Factor-Alpha", "Interferon-Gamma"

Inclusion Criteria	Exclusion Criteria	Searched Databases	Study Selection Approach
Studies investigating the relationship between eosinophils and cognitive impairment in individuals diagnosed with schizophrenia.	Studies not focused on schizophrenia or cognitive impairment.	PubMed	Screening of titles and abstracts for relevance.
Studies published in peer-reviewed journals.	Studies conducted solely on animal models.	Scopus	Assessment of full-text articles against inclusion and exclusion criteria.
Studies with human subjects of any age, gender, or ethnicity.	Studies not available in English.	Good Scholar	Data extraction from selected studies.
Studies available in English.	Case reports, reviews, letters, commentaries, and editorials.	Embase	Quality assessment of included studies.
Studies employing observational, experimental, or interventional designs.	Studies lacking clear methodology or results.	Web of Science	Synthesis of findings through narrative or meta-analysis if appropriate.
Studies that assess eosinophil levels through blood tests or other reliable methods.	Studies with insufficient data for analysis.	Cochrane Library	
Studies that measure cognitive impairment using standardized neuropsychological tests or clinical assessments.	Duplicate publications or redundant data.		

Schizophrenia and Cognitive Impairment

Schizophrenia is a complex and debilitating psychiatric disorder that is often characterized by a range of cognitive impairments. Cognitive impairment in schizophrenia can significantly impact an individual's daily functioning, quality of life, and overall outcomes.[9–11] People with schizophrenia often experience difficulties in working memory, which involves holding and manipulating information for short periods. This can hinder problem-solving and decision-making abilities.[12] Impaired attention and concentration are common, making it challenging for individuals to focus on tasks or filter out irrelevant information. Executive functions, including planning, organization, and decision-making, are often impaired, affecting a person's ability to set and achieve goals. Verbal memory impairments can affect the ability to remember and understand spoken information. Some individuals with schizophrenia may struggle with visual-spatial processing, impacting tasks that require spatial awareness.[13] Cognitive processing speed is often slower in individuals with schizophrenia, affecting the speed at which they can complete tasks and respond to stimuli.[14] Structural and functional brain abnormalities, such as reduced gray matter volume and altered connectivity, are thought to play a role in cognitive impairment. Dysregulation of neurotransmitters, including dopamine and glutamate, may contribute to cognitive deficits in schizophrenia.[15] Immune system dysregulation and neuroinflammation have also been implicated in cognitive impairment in schizophrenia.[16] Genetic predisposition and environmental stressors can further influence cognitive function in schizophrenia.[17] Some antipsychotic medications may have cognitive side effects, although newer medications aim to minimize these effects. Cognitive impairment in schizophrenia can impact various aspects of daily life, including employment, social relationships, and independent living. Individuals with schizophrenia may find it challenging to complete educational or vocational training, hold down a job, manage finances, or engage in social activities.[18] Comprehensive neuropsychological assessments can help identify specific cognitive deficits in individuals with schizophrenia. Cognitive remediation therapies, psychoeducation, and rehabilitation programs are often used to improve cognitive function and functional outcomes. Medications that target the underlying symptoms of schizophrenia can also indirectly benefit cognitive function.[19] Ongoing research aims to better understand the precise neural mechanisms underlying cognitive impairment in schizophrenia and to develop targeted interventions. Cognitive impairment is a significant aspect of schizophrenia that poses challenges for both individuals living with the condition and healthcare professionals. Addressing these cognitive deficits is an important component of comprehensive care and treatment for individuals with schizophrenia.[20]

The Immunological Basis of Schizophrenia

The immunological basis of schizophrenia is an evolving area of research that suggests a complex interplay between the immune system and the development or exacerbation of schizophrenia.[21] While the exact mechanisms are not fully understood, growing evidence points to immune system dysregulation and inflammation as contributing factors in the pathogenesis of this psychiatric disorder. Studies have shown that individuals with schizophrenia often exhibit higher levels of pro-inflammatory markers, such as cytokines, in their blood and cerebrospinal fluid. These markers are indicative of immune system activation and inflammation.[22,23] Microglia, the resident immune cells in the brain, can become activated in response to infection, stress, or injury. This activation may contribute to neuroinflammation and affect brain function.[24] There is some evidence of autoimmune processes being involved in schizophrenia. Autoantibodies targeting brain proteins have been detected in some individuals with the disorder.[25] Some research has suggested that exposure to infections during prenatal development or childhood may increase the risk of developing schizophrenia later in life. This may be linked to maternal immune responses or the child's immune system.[26] Psychological stress can also lead to immune activation and the release of pro-inflammatory cytokines.[27] Stressful life events have been associated with an increased risk of developing schizophrenia.[28] The blood-brain barrier (BBB) separates the bloodstream from the brain and spinal cord. Disruption of the BBB may allow immune cells and molecules to enter the brain, potentially leading to neuroinflammation.[29] Chronic neuroinflammation may contribute to neurodegeneration and structural brain changes in individuals with schizophrenia.[30] It's important to note that while immune system dysregulation and inflammation are associated with schizophrenia, they are not the sole causative factors. The development of schizophrenia is likely influenced by a combination of genetic, environmental, and immunological

factors.[31] The understanding of the immunological basis of schizophrenia has led to the exploration of potential therapeutic interventions targeting the immune system or inflammation.[32] Some clinical trials are investigating the use of anti-inflammatory drugs as adjunctive treatments for schizophrenia. Research in this area is ongoing, and scientists continue to investigate the precise mechanisms through which the immune system and inflammation may contribute to the development and progression of schizophrenia. It's important to emphasize that while the immunological basis of schizophrenia is a promising avenue for research and potential treatments, it is just one facet of a multifactorial disorder. The exact role of the immune system and how it interacts with other genetic and environmental factors in schizophrenia remain areas of active investigation and debate in the scientific community.

Eosinophils: Form and Function

Eosinophils are a type of white blood cell, specifically a granulocyte, that plays a crucial role in the immune system. They are characterized by their distinct bi-lobed nucleus and granules within the cytoplasm that stain bright red or orange when exposed to certain dyes, such as eosin, hence the name eosinophils.[33] Eosinophils are particularly effective in combating parasitic infections, such as helminths (worms) and certain protozoa. They release cytotoxic substances, including enzymes and proteins like major basic protein (MBP) and eosinophil peroxidase, which can kill parasites or limit their growth.[34] Eosinophils play a role in allergic reactions and asthma. When an allergic reaction occurs, the immune system releases substances that attract eosinophils to the site of inflammation. These cells then release their granule contents, contributing to tissue damage and inflammation in allergic conditions.[35] Eosinophils also participate in modulating inflammatory responses. They release cytokines, which are signaling molecules that regulate the activity of other immune cells, contributing to the overall immune response. In response to stimuli, eosinophils may release a range of granule proteins, including major basic proteins (MBPs) 1 and 2, eosinophil cationic protein (ECP), eosinophil peroxidase (EPX), eosinophil derived neurotoxin (EDN), cytokines, and cytosolic Charcot-Leyden crystal protein/galectin-10 (CLC/Gal-10) among others.[36] While eosinophils are known for their role in host defense and inflammation, they also have roles in tissue repair and remodeling. They can produce growth factors that aid in tissue regeneration and repair after injury or inflammation. Although eosinophils are essential for the body's defense against certain infections and for managing allergic reactions, abnormal levels of eosinophils can indicate underlying health issues including eosinophilic granulomatosis with polyangiitis (EGPA), eosinophilic esophagitis (EOE), and hypereosinophilic syndrome (HES) Furthermore, eosinophilic dysfunction might be indicative of various conditions, including allergies, parasitic infections, autoimmune diseases, or certain cancers.

Eosinophils in Schizophrenia and Cognitive Impairment

The involvement of eosinophils in schizophrenia and cognitive impairment is an area of ongoing research, although the specific role of eosinophils in these conditions is not yet fully understood. Schizophrenia is a complex mental disorder characterized by disturbances in thinking, perception, emotions, and behavior. Cognitive impairment often accompanies schizophrenia and can manifest as deficits in memory, attention, and executive function. Eosinophils, being part of the immune system, have garnered attention in this context due to their involvement in inflammatory responses and immune modulation.[37] For example, T cell dysfunction has been reported in schizophrenia[38] and eosinophils have the ability to regulate T cell function with respect to polarization of T cells to either the Th2 or Th1 pathway in that that they express both Th1- and Th2-associated cytokines.[37] Elevated levels of certain eosinophil-related cytokines and immune cells have been reported in some studies. These findings suggest a possible association between inflammation and the pathophysiology of schizophrenia due to modulation by eosinophils. Eosinophils might contribute to neuroinflammation, potentially impacting brain function and cognitive processes by influencing integrity of the BBB.

Emerging Research on Eosinophils in Schizophrenia

Emerging research on eosinophils in schizophrenia is shedding light on the potential role of these white blood cells in the pathogenesis of the disorder. While the field is still evolving, several recent studies and findings have provided new insights into the connection between eosinophils and schizophrenia.[32] Some studies have reported alterations in eosinophil counts in individuals with schizophrenia. While the findings are not consistent across all studies, the variations

suggest the potential involvement of eosinophils in the immune response in schizophrenia.[39,40] Eosinophils are traditionally associated with allergic and inflammatory responses.[41] Emerging research suggests that immune system dysregulation and inflammation are linked to the pathophysiology of schizophrenia. Altered cytokine levels in schizophrenia have been widely reported, and eosinophils may be a source of these cytokines.[42,43] Eosinophils are also implicated BBB disruption which may permit immune cells, including eosinophils, to enter the brain, leading to neuroinflammation, which is associated with schizophrenia.[44] Research is starting to explore different subtypes of eosinophils, each with distinct functions. These subtypes may have varying roles in the immune response and may be differentially regulated in individuals with schizophrenia. Studies by Cabrera Lopez et al have identified differences in eosinophil surface proteins as either 1) resident eosinophils (Siglec-8^{+}CD62L^{+}IL-$3R^{lo}$) or 2) inflammatory eosinophils (iEos; Siglec-8^{+}CD62L^{lo}IL-$3R^{hi}$) via flow cytometry and confocal microscopy.[45] Eosinophils do not work in isolation. They interact with other immune cells, such as microglia and T cells, which are also implicated in the immune response in schizophrenia. Emerging research is beginning to examine these complex interactions. Understanding the role of eosinophils in schizophrenia may have therapeutic implications. Targeting eosinophils or related pathways could potentially lead to novel treatments for the disorder. Emerging research in this area faces several challenges, including the need for larger and more comprehensive studies, as well as the elucidation of the specific mechanisms by which eosinophils contribute to schizophrenia. Future research will likely focus on clarifying these aspects. As the understanding of eosinophils in schizophrenia continues to evolve, it holds the promise of providing new perspectives on the immune system's involvement in the disorder and potential avenues for the development of targeted interventions. Further studies and collaboration between researchers in immunology and psychiatry are essential to fully uncover the role of eosinophils in the complex landscape of schizophrenia.

Mechanisms and Pathways of Eosinophils in Schizophrenia and Cognitive Impairment

The mechanisms and pathways through which eosinophils may be involved in schizophrenia and cognitive impairment are still the subject of ongoing research.[46] While our understanding is not yet complete, emerging evidence suggests several potential mechanisms and pathways that may link eosinophils to cognitive impairment in individuals with schizophrenia. Eosinophils are known to produce cytokines, such as interleukins (eg, IL-4 and IL-5) and chemokines.[47] These cytokines can modulate the immune response and have the potential to affect neural function. In schizophrenia, an imbalance of cytokines, particularly pro-inflammatory cytokines, has been observed.[48] Eosinophil-derived cytokines may contribute to this imbalance, leading to inflammation in the brain, which is associated with cognitive impairment. Eosinophils are involved in immune responses and can potentially migrate to sites of inflammation, including the brain.[49] The disruption of the BBB, which separates the bloodstream from the brain, can allow immune cells, including eosinophils, to enter the brain. This migration may lead to neuroinflammation, affecting neural pathways and cognitive function.[44] Eosinophils, when activated, release inflammatory molecules, and they may contribute to the overall neuroinflammatory response in individuals with schizophrenia.[50] Chronic neuroinflammation has been associated with structural brain changes and cognitive deficits. Eosinophils can produce neurotransmitters like serotonin. Alterations in serotonin signaling have been implicated in schizophrenia and cognitive dysfunction.[51] Eosinophil-derived neurotransmitters may influence neurotransmitter imbalances in the brain. Eosinophils do not operate in isolation. They interact with other immune cells, such as microglia (the brain's resident immune cells), T cells, and astrocytes. The interplay between eosinophils and other immune cells may contribute to the immune response in the brain, affecting cognitive function. Autoimmune processes may be involved in schizophrenia, and eosinophils could play a role in this context. Autoantibodies targeting brain proteins have been identified in some individuals with schizophrenia.[52] Eosinophils may contribute to these autoimmune responses.

Research into different subtypes of eosinophils may reveal distinct functions. Some subtypes may be more pro-inflammatory, while others may have anti-inflammatory properties. Understanding the balance of eosinophil subtypes and their regulation in the context of schizophrenia and cognitive impairment is an area of interest. Genetic factors, such as genetic predisposition to immune dysregulation, and environmental stressors may interact with eosinophil-related

mechanisms, contributing to cognitive deficits in individuals with schizophrenia.[53,54] Although some reports have not shown differences in blood eosinophil concentrations in patients with schizophrenia, compared with healthy donors, Hallgren et al have reported elevated serum levels of lactoferrin and eosinophil cationic protein in schizophrenic patients[55] and others have reported a significant increase in blood eosinophil levels in patients with schizophrenia. Further, eosinophils were the only blood cells that were significantly reduced in women with schizophrenia compared to men with schizophrenia. Whether or not elevated eosinophils modulate neuro-cognitive function in schizophrenia in a manner similar to other diseases of cognitive dysfunction such as eosinophilia-myalgia syndrome,[56] remains to be determined.

In contrast, in those studies there were no differences in basophils in patients with schizophrenia compared with healthy controls.[38] Interestingly, elevated eosinophils have been associated with improved nerve growth or cognition in other studies. In a murine model of atopic dermatitis, eosinophils dramatically increased branching of sensory neurons isolated from the dorsal root ganglia (DRG) of the experimental mice suggesting a pathophysiological role for eosinophils in cutaneous nerve growth in atopic dermatitis.[57] In addition, although there have been reports of cognitive impairment with verbal learning and memory in over half of patients with severe eosinophilic asthma,[58] there are eosinophilic asthma patients who remain cognitively intact suggesting that there are other factors that likely modulate cognitive function in the presence of eosinophils. Furthermore, elevated eosinophils are well described in the arena of opportunistic and other infections[59] and do not always present with cognitive concerns, suggesting that there are likely multiple factors that need to be engaged to foster cognitive decay in the presence of hyper-eosinophilia. It's important to note that the exact contribution of eosinophils and the mechanisms involved are still being investigated. As research in this area progresses, a more comprehensive understanding of how eosinophils may impact cognitive impairment in schizophrenia will likely emerge. Further studies are needed to elucidate the specific roles and interactions of eosinophils within the complex immune and neural systems in individuals with schizophrenia.

Therapeutic Implications

The clinical implications and therapeutic potential of eosinophils in schizophrenia are areas of growing interest and research. Investigating the relationship between eosinophils and cognitive impairment in schizophrenia may have therapeutic implications. If eosinophils are found to play a significant role in mediating inflammation and cognitive dysfunction in schizophrenia, targeting eosinophilic inflammation could represent a novel therapeutic approach. This could involve repurposing existing anti-inflammatory drugs or developing new treatments specifically targeting eosinophils or their downstream inflammatory pathways.

While the field is still in its early stages, understanding the role of eosinophils in schizophrenia may have important implications for diagnosis, treatment, and the development of novel therapeutic strategies.[60] Eosinophil levels and activation status may serve as potential biomarkers to distinguish different subtypes of schizophrenia. This could aid in tailoring treatment approaches to individual patients based on their immune profiles.[61] Eosinophil profiles and their interaction with other immune markers could potentially be used to predict disease progression or treatment response in individuals with schizophrenia.[58] Eosinophils might offer insights into early stages of schizophrenia development, allowing for early detection and intervention, which could improve treatment outcomes.[62] Understanding the eosinophilic involvement in schizophrenia may help identify individuals at higher risk of developing comorbidities related to immune dysregulation and inflammation, including autoimmune disorders.

Targeting eosinophils or modulating their activity may offer a novel therapeutic approach for individuals with schizophrenia. Immune-modulating therapies, such as anti-inflammatory medications, may help reduce neuroinflammation and potentially improve cognitive function.[63,64] By considering the eosinophil profile of individuals with schizophrenia, personalized treatment plans can be developed. Some patients may benefit from immune-targeted therapies, while others may require different interventions. Eosinophil-targeted treatments could be used as adjunctive therapies alongside standard antipsychotic medications to address specific aspects of the disorder, such as cognitive impairment and inflammation.[65] Therapies aimed at mitigating eosinophil-related neuroinflammation could have neuroprotective effects, potentially preventing or minimizing structural brain changes associated with schizophrenia. The emerging field of psychoneuroimmunology focuses on understanding the connections between the immune system and mental health.

Incorporating eosinophil-related research into this framework could lead to innovative treatment strategies. Targeted interventions based on eosinophil research could be designed to address cognitive deficits associated with schizophrenia, potentially enhancing cognitive function and quality of life.[66] It's important to emphasize that research into the therapeutic potential of eosinophils in schizophrenia is ongoing, and more studies are needed to validate the efficacy and safety of these approaches. Additionally, personalized treatment plans should take into account each patient's unique immunological profile and needs. As the field continues to evolve, it holds promise for providing new insights and treatment options for individuals with schizophrenia.

Future Directions of Eosinophils in Schizophrenia

The study of eosinophils in schizophrenia is an evolving field, and future research directions hold the promise of further elucidating the role of eosinophils in the disorder.[67] Research may delve deeper into understanding the various subtypes of eosinophils and their specific functions in the context of schizophrenia. Investigating the roles of distinct eosinophil subpopulations could provide insights into their differential impact on the disorder. Ongoing research may aim to identify eosinophil-related biomarkers that can be used for diagnostic and prognostic purposes in schizophrenia. The discovery of specific eosinophil-related markers may help in patient stratification and personalized treatment approaches. Additionally, elucidating the underlying mechanisms linking eosinophils to cognitive impairment may pave the way for the development of personalized treatment strategies targeting specific immune pathways implicated in schizophrenia.

It is important to caution that it is necessary to consider the influence of other acquired factors such as drugs (ie clozapine) and deleterious habits (ie smoking) as well as innate genetic polymorphisms that may further affect the relationship of eosinophils in schizophrenia. Future studies should investigate the causal relationship between eosinophils and schizophrenia. This may involve longitudinal research to determine whether eosinophil activity precedes the onset of schizophrenia or is a consequence of the disorder. Research can explore the intricate interactions between eosinophils and other immune cells, such as microglia, T cells, and astrocytes. Understanding these interactions and their implications for schizophrenia pathogenesis is critical. Further investigation into the potential autoimmune processes in schizophrenia, including the role of eosinophils in producing autoantibodies against brain proteins, is needed. Studies may focus on the interplay between genetic predisposition and environmental factors in eosinophil-related mechanisms, potentially revealing why some individuals with certain genetic backgrounds are more susceptible to eosinophil-related immune dysregulation in schizophrenia. Future research could explore the precise mechanisms through which eosinophils contribute to neuroinflammation and cognitive impairment in schizophrenia. This may involve both in vitro and in vivo studies. Clinical trials and experimental therapies that target eosinophil-related pathways could be developed and tested for their efficacy in improving cognitive function and overall outcomes in individuals with schizophrenia. Based on eosinophil-related markers and profiles, researchers may work toward identifying subgroups of individuals with schizophrenia who are more likely to benefit from specific treatment strategies, including immune-modulating therapies. Collaboration between researchers in immunology, psychiatry, neuroscience, and other related fields is essential to gain a comprehensive understanding of the role of eosinophils in schizophrenia. Multidisciplinary approaches can help connect findings from different areas of expertise. As the field of eosinophils in schizophrenia research progresses, it will be important to integrate findings from these future directions into a broader understanding of the disorder. By providing a posit for novel thought in this vexing disease space, additional collaboration, innovative research methods, and a focus on personalized medicine may help uncover new insights and therapeutic strategies for individuals with schizophrenia.

Clinical and Health Implications

The potential involvement of eosinophils and immune dysregulation in conditions like schizophrenia and cognitive impairment could have significant implications for health policy makers:

Research Funding Allocation

Encouraging and supporting research initiatives focused on understanding the role of eosinophils and the immune system in schizophrenia and cognitive impairment is crucial. Health policy makers can allocate funding toward studies investigating the mechanisms underlying immune dysregulation in these conditions. This could include supporting interdisciplinary research involving immunologists, neuroscientists, psychiatrists, and other relevant fields.

Integrated Healthcare Approaches

Policy makers could advocate for integrated healthcare approaches that consider the interaction between the immune system and mental health. This might involve promoting collaboration between mental health professionals and immunologists to develop comprehensive treatment strategies that target both neurological and immune aspects of these disorders.

Early Detection and Intervention

Supporting initiatives for early detection and intervention of immune-related mechanisms in schizophrenia and cognitive impairment could improve patient outcomes. Policy makers can facilitate the development of screening programs and diagnostic tools that assess immune biomarkers, including eosinophil-related markers, to identify individuals at risk or in the early stages of these conditions.

Treatment Strategies

Understanding the role of eosinophils and immune pathways may lead to the development of novel treatment strategies. Health policy makers can prioritize the evaluation and potential implementation of therapies targeting immune dysregulation, such as immunomodulatory treatments, alongside traditional approaches for managing schizophrenia and cognitive impairment.

Education and Awareness

Increasing awareness among healthcare professionals, policymakers, and the general public about the potential link between the immune system and mental health disorders like schizophrenia could lead to improved recognition, diagnosis, and management of these conditions. Policy makers can support educational campaigns to disseminate information about emerging research findings in this area.

Ethical Considerations and Regulations

Policy makers also play a role in establishing ethical guidelines and regulations for the development and implementation of new treatments targeting immune pathways. This includes ensuring patient safety, informed consent, and ethical considerations surrounding the use of innovative therapies in mental health care.

Conclusion

The exploration of eosinophils and their potential involvement in cognitive impairment in schizophrenia marks a new and promising perspective in the field of neuropsychiatry. While our understanding is still in its infancy, the emerging research offers a fresh outlook on the complex interplay between the immune system and the cognitive deficits that characterize this enigmatic disorder. Eosinophils, traditionally associated with allergic responses and inflammation, are now being considered as potential contributors to the intricate immune dysregulation observed in individuals with schizophrenia. The mechanisms through which eosinophils may influence cognitive function are multifaceted and interconnected, involving cytokine production, neuroinflammation, blood-brain barrier disruption, and complex interactions with other immune cells.

This new perspective holds potential clinical implications, as it posits towards the putative application for eosinophil profiles to serve as biomarkers, predictive indicators, and tools for personalized treatment in schizophrenia. Furthermore, the therapeutic potential of targeting eosinophil-related pathways offers hope for innovative interventions that may

alleviate cognitive impairment and enhance the quality of life for those affected by the disorder. It is essential to recognize the ongoing nature of this research. While the role of eosinophils in schizophrenia and cognitive impairment has been illuminated, numerous questions remain unanswered. Future investigations will need to delve deeper into the specifics of eosinophil subtypes, the causality and temporality of their involvement, and their interaction with other immune cells. The collaboration between researchers from diverse fields—immunology, psychiatry, neuroscience, and beyond—will be pivotal in advancing our understanding of the role of eosinophils in schizophrenia. The potential for a more nuanced and personalized approach to diagnosis and treatment hinges on the collective efforts of the scientific community.

Disclosure

The authors report no conflicts of interest in this work.

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REVIEW

Real-World Evidence of Relapsed/Refractory Mantle Cell Lymphoma Patients and Treatments: A Systematic Review

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Introduction: Mantle cell lymphoma (MCL) is an incurable disease with an aggressive clinical course, and most patients eventually relapse after chemotherapy. Targeted therapies developed for relapsed/refractory MCL have been approved based on clinical trial data. However, real-world setting data are scarce and scattered.

Areas Covered: This systematic review aimed to collect, synthesize, and describe the characteristics and treatment outcomes of patients with relapsed/refractory MCL after receiving a second or subsequent line of therapy in the real-world setting.

Expert Opinion: R/R MCL is clinically and biologically heterogeneous and still represents a therapeutic challenge, with high-risk and early relapsed patients remaining an unmet medical need. This systematic review is limited by the quality of the available data and the difficulty of comparing outcomes in R/R MCL due to the heterogeneity of the disease, but the results suggest that covalent BTKis should be positioned as second-line therapy, followed by CAR T-cells in BTK-i-relapsed patients. Chemo-free and combination therapies with established chemoimmunotherapy backbones in the relapsed and front-line settings have been recently developed, and front-line options are being improved to move targeted and cellular therapies to earlier lines, including front-line therapy, in elderly and younger fit patients. In the upcoming years, many new targeted agents will play an important role and will be incorporated to the routine practice as their sequence, and outcomes in unselected patients are determined.

Keywords: CAR-T cells, ibrutinib, mantle cell lymphoma, real-world evidence, relapsed/refractory mantle cell lymphoma (R/R MCL), treatment efficacy

Introduction

Mantle cell lymphoma (MCL) is an infrequent subtype of non-Hodgkin lymphoma (NHL) that accounts for approximately 5 to 7% of lymphoid malignancies in Western Europe,[1] but its incidence seems to be increasing over time.[2] It is an incurable disease with a median age at diagnosis of 68 years,[3] more common in men than in women (ratio around 3:1).[1,2]

Although two types of clinically indolent MCL variants have been recognized —leukemic non-nodal MCL and in situ mantle cell neoplasia— most patients with MCL present with an aggressive clinical course.[1,4] Moreover, MCL patients usually experience multiple relapses, and survival outcomes worsen with increasing lines of therapy.[5] Some clinical and pathological features have been identified as prognostic factors of MCL, such as the MCL International Prognostic Index (MIPI), the Ki-67 index, aberrations in the TP53 tumor suppression gene (eg, TP53 mutations and del17p), presence of blastoid or pleomorphic histologic variants, and an early progression of disease after first-line therapy, especially within the first one or two years.[6]

Historically, several chemotherapy-based strategies have been used for MCL depending on the patient's age, functional status, and number of previous lines of therapy, but most patients eventually relapse.[1] In the last few years, several targeted treatment approaches have been developed for relapsed/refractory MCL (R/R MCL), including Bruton's tyrosine kinase (BTK) inhibitors, B-cell lymphoma 2 (BCL-2) inhibitors, lenalidomide and bortezomib-based approaches, m-TOR inhibitors, and chimeric antigen receptor (CAR) T-cell therapy.[6] Of those, the first–in–class BTK inhibitor ibrutinib has been positioned

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as the standard of care in the second line of therapy for MCL, based on the data from a pooled analysis of three clinical trials of R/R MCL patients treated with ibrutinib.[7]

The recommendations of treatment guidelines for R/R MCL are usually based on clinical trial data.[1] Nevertheless, it is considered useful to validate the efficacy and safety of treatments in real-world studies to adopt them in routine clinical practice. In this regard, the real-world evidence currently available on R/R MCL treatments is scarce and scattered,[8–14] and it is often difficult to compare due to the diversity of the approaches and the patients' characteristics. Therefore, this systematic review aimed to collect, synthesize, and describe the characteristics and treatment outcomes of patients with R/R MCL after receiving a second or subsequent line of therapy in the real-world setting.

Materials and Methods

A systematic literature search was performed and reported in compliance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement, a guideline for standard reporting of systematic literature reviews.[15]

Eligibility Criteria

Real-world studies including patients with confirmed R/R MCL, written in English, published between 2010 and 2022, and indexed in PubMed or corresponding to 2021 congress publications of the American Society of Clinical Oncology (ASCO), the American Society of Hematology (ASH), the European Hematology Association (EHA), or the International Conference on Malignant Lymphoma (ICML) were eligible for inclusion. Conversely, clinical trials, case studies, or case series of <10 patients (excepting those considered relevant to mention in this review due to their uniqueness), and publications not reporting original data (eg, letters, editorials, comments, or systematic reviews) were excluded from the study. Other exclusion criteria were patients naïve to MCL treatment, outdated treatment regimens, and studies not reporting outcomes regarding survival or treatment response. Moreover, we excluded studies assessing transplantation strategies, given that they are mostly consolidation therapies with outcomes depending on previous rescue treatments, precluding direct comparisons.

Information Sources and Search Strategy

The literature search was performed on 15 May 2022 using the MEDLINE database through PubMed and websites with relevant conference materials on the subject —ie, the proceedings of the biennial International Conference on Malignant Lymphoma (ICML)[16] and the annual meetings of the American Society of Hematology (ASH),[17] the American Society of Clinical Oncology (ASCO),[18] and the European Hematology Association (EHA).[19]

The search strategy in MEDLINE was as follows: ("relapsed" AND/OR "refractory") AND "mantle cell lymphoma" AND ("retrospective" OR "real life" OR "real world" OR "case report"). In addition, for the manual search of ICML, ASH, ASCO, and EHA conference proceedings, the keywords "relapsed", "refractory", mantle cell lymphoma, "real world", "retrospective", "real life", and "case series" were used.

In order to cover the most relevant pharmacological strategies for R/R MCL in current daily clinical practice, the search was limited to articles written in English, with full text available, published between 2010 and 2022. Furthermore, if the most relevant results presented to international congresses before 2021 would have already been published as journal articles, the abstracts of the proceedings were manually searched only for studies published since 2021.

Study Selection, Data Collection, and Data Items

Two reviewers independently screened all titles/abstracts and the full text of the retrieved publications potentially relevant for inclusion. Any disagreements were resolved by consulting with a third author. Similarly, the data were independently collected by two reviewers in predefined table disagreements, if any, were resolved by discussion with a third author.

The data collected related to the study included the first author, the year of publication, the treatment, and the sample size. Data regarding the baseline characteristics of study patients included age, gender, Eastern Cooperative Oncology Group (ECOG) score, stage of the disease according to the Ann-Arbor classification, number of previous lines of therapy,

previous autologous or allogeneic stem cell transplantation (auto-SCT or allo-SCT, respectively), refractoriness to previous lines of therapy, progression of disease within 24 months (POD24), MIPI or simplified MIPI (sMIPI), Ki-67 index, TP53 aberrations, and presence of aggressive histologic variants (ie, blastoid or pleomorphic MCL). Clinical outcomes related to treatment efficacy were as follows: progression-free survival (PFS), overall survival (OS), overall response rate (ORR), complete response (CR), and follow-up.

Risk of Bias Assessment

The risk of bias of the included studies was assessed by two reviewers using the Joanna Briggs Institute (JBI) critical appraisal tool for case series.[20] Any discrepancies were resolved by discussion with a third author.

Data Synthesis and Analyses

Data were presented as a narrative synthesis of the available data reported for each retrieved treatment regimen.

Results

A total of 300 publications were identified using the described search strategies. After removing all duplicates and excluding studies that did not meet the inclusion criteria, a total of 25 journal articles —18 original articles,[8–11,14,21–33] 3 letters to the editor,[34–36] 3 short reports,[13,37,38] and 1 case report[39] and 5 conference publications[40–44] were included in the systematic review. All the 30 studies allowed the data collection of 37 treatment regimens for R/R MCL patients. Of them, 15 were based on BTK inhibitors, 14 on chemotherapy or immunochemotherapy, and 8 on other strategies. Table 1 summarizes the general characteristics of the treatment regimens and patients included in the systematic review.

BTK Inhibitor Regimens

All studies on BTK inhibitors analyzed the use of ibrutinib, either as monotherapy (n = 13 treatment regimens)[8–11,14,21–24,34,37,40,41] or combined (n = 2).[24,39]

Ibrutinib Monotherapy

The treatment regimens based on ibrutinib monotherapy included from 33 to 211 patients (n = 12), mostly males (65% to 82%, n = 12), with a median age at treatment between 65 and 74 years (n = 8) (Table 1). The percentages of patients with an ECOG score ≥2 and a III–IV stage R/R-MCL according to the Ann-Arbor classification ranged from 5.2% to 34% (n = 9) and from 68.1% to 93% (n = 10), respectively. The median number of previous lines of therapy varied from 1 to 3 (n = 10), and the proportion of patients with a previous auto-SCT was between 13% and 66% (n = 11), and between 0% and 11% (n = 4), for allo-SCTs. Regarding response to previous treatments, between 16% and 48.1% of the patients were refractory to first-line therapy (n = 3) and between 18.2% and 47.1% were refractory to the most recent treatment line (n = 3); 47.8% to 54% of the patients were POD24 regarding their front-line therapy (n = 3). Additionally, the percentage of patients with an intermediate-high MIPI/sMIPI and a Ki-67 index ≥30% ranged between 44% and 87% (n = 7), and between 33.3% and 55.6% (n = 5), respectively. The proportion of patients presenting with high-risk blastoid or pleomorphic histology differed considerably among studies (from 3.4% to 32.6%, n = 9), with only three studies reporting TP53 aberrations, which ranged from 0 to 20%.

The efficacy outcomes of patients treated with ibrutinib alone were also quite variable and included a median PFS ranging from 7.9 to 30.8 months (n = 13), a median OS from 12.4 to 38 months (n = 12), an ORR from 36.4% to 95.9% (n = 10), and a CR from 15% to 39.5% (n = 9), with median follow-ups ranging from 12.6 to 60 months (Table 2).

Ibrutinib in Combination

Two studies analyzed the efficacy of ibrutinib in combination with other agents. The first one reported the outcomes of ibrutinib combined with several other agents (rituximab, lenalidomide, bortezomib, and/or bendamustine) in 53 patients (75.5% males, median age of 56 years),[24] of which 28.3%, 84.9%, 51.0%, and 54.7% had an ECOG score ≥2, a III–IV stage R/R-MCL, an intermediate-high sMIPI, and a Ki-67 index ≥30%, respectively. In addition, the percentages of patients with a previous auto-SCT, refractoriness to the most recent line of therapy, and blastoid histology were 9.4%, 47.2%, and 19.2%.

Table 1 General Characteristics of the Assessments and Patients Included in the Systematic Review According to the Type of R/R MCL Treatment[a]

First author, publication date	Treatment	Patients, N	Age (years), *median (range)*	Male gender, n (%)	ECOG score ≥2, n (%)	Ann-Arbor stages III–IV, n (%)	No. of previous LoT, *median (range)*	Previous auto-SCT, n (%)	Previous allo-SCT, n (%)	Refractoriness to previous LoT, n (%)	POD24, n (%)	MIPI or sMIPI intermediate–high, n (%)	Ki-67 index ≥30%, n (%)	TP53 aberrations n (%)	Blastoid or pleomorphic histology, n (%)
BTK inhibitors															
Ibrutinib monotherapy															
Broccoli 2018[10]	Ibrutinib	77	65.2 (34.6–81.3)	59 (76.6)	16 (20.7)	69 (89.6)	3 (1–10)	27 (35)	NR	37 (48.1) to 1LT; 17 (22.1) to most recent LoT	NR	NR	NR	NR	3 (3.9), blastoid
Yi 2021[34]	Ibrutinib	88	71 (42–92)	71 (80.7)	8 (9.1)	77 (87.5)[b]	1 (1–6)	12 (13.6)	NR	NR	NR	70 (79.5)	*median (range): 35.0 (10.0–95.0)*[b]	0 (0.0)[b], TP53/del(17p)	3 (3.4), blastoid[b]
McCulloch 2021[11]	Ibrutinib	211	73 (33–96)	147 (70)	46 (24)	194 (93)[b]	1 (1–1)	50 (24)	3 (1)	NR	109 (52) with 1LT	123 (87)	76 (54)[b]	NR	29 (14), blastoid[b]
Tucker 2021[37]	Ibrutinib	65	67 (48–90)	76%[c]	34%[c]	NR	2 (1–6)	Approx. 66%[c]	NR	NR	NR	0 (0)	NR	NR	18%, blastoid[c]
Jeon 2019[21]	Ibrutinib	33	65 (40–79)[b]	27 (81.8)	4 (12.1)[d]	28 (84.9)[b]	33% 1 prior LoT*	6 (18.2)	0 (0.0)	NR	NR	19 (57.6)[d]	11 (33.3)[d]	NR	NR
Epperla 2017[14]	Ibrutinib	97	63 (39–87)[b]	80 (82)	14 (14)[b]	88 (91)[b]	2 (1–8)	38 (39)	11 (11)	7 (7) primary refractory disease	NR	43 (44)[b]	37 (38)[b]	NR	15 (16), blastoid[b]
Visco 2021[9]	Ibrutinib	50	58 (19–70)[b]	37 (74.0)	NR	NR	1 (1–1)	23 (46)	NR	8 (16) to 1LT	27 (54) with 1LT	*High: 16 (33)*[d]	NR	NR	20 (20)[d]
Sancho 2022[22]	Ibrutinib	66	69.3 (60.9–76.2)	52 (78.8)	0–1: 59 (93.7)[d]	61 (92.4)[d]	2 (1–7)	14 (21.2)	NR	12 (18.2) to most recent LoT	12 (18.2)	42 (63.6)[b]	20 (55.6)[b]	2 (9.1)[d], TP53/del(17p)	12 (24.5), blastoid; 4 (8.2), pleomorphic[b]
Sharman 2021[23]	Ibrutinib 2L+3L	117	*2nd LoT: 71.6 (48.2–>90); ≥3rd LoT: 68.5 (53.3–88.3)*	117 (79.6)	21 (14.3)	129 (87.8)[b]	NR	NR	NR	NR	NR	NR	NR	NR	NR
Cencini 2021[8]	Ibrutinib	69	70 (41–89)[d]	45 (65.2)	NR	47 (68.1)	1 (1–4)	9 (13.0)	1 (1.5)	12 (17.4) to 1LT	33 (47.8) with 1LT	*High: 21 (30.4)*	NR	NR	7 (10.2)[d]
Janssens 2021[40]	Ibrutinib	76	74.0 (47.0–88.0)	58 (76.3)	3 (5.2)[d]	NR	1 (1–3)	NR	NR	NR	NR	NR	NR	2 (20)[d], TP53/del(17p)	NR
Obr 2021[41]	Ibrutinib	77	68 (40–81)[b]	NR	NR	80%[c,d]	2 (1–8)	24.7%[c,d]	NR	NR	61%[c,e]	84.7%[c,d]	NR	NR	NR
Zhang 2022[24]	Ibrutinib	68	63 (34–81)	45 (66.2)	15 (22.1)	61 (89.7)	60.3% 1 prior LoT;	3 (4.4)	NR	32 (47.1) to most recent LoT	NR	42 (61.8)	31 (45.6)	NR	7 (11.5), blastoid

Ibrutinib in combined therapy															
Zhang 2022[24]	Ibrutinib + Other agents	53	56 (42–80)	40 (75.5)	15 (28.3)	45 (84.9)	66% 1 prior LoT	5 (9.4)	NR	25 (47.2) to most recent LoT	NR	27 (51.0)	29 (54.7)	NR	10 (19.2), blastoid
Fabbri 2020[39]	Ibrutinib + Venetoclax	4	47 (40–59)[b]	3 (75.0)	0 (0.0)[d]	IV: 4 (100)[d]	NR	NR	NR	4 (100) to 1LT and most recent LoT	NR	*High: 3 (75)*[d]	4 (100)[d]	NR	3 (75)[d]
Chemotherapy/immunochemotherapy															
Bendamustine-based approaches															
Rigacci 2012[25]	Bendamustine ± R	30	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Warsch 2012[26]	Bendamustine ± R	25	NR	NR	NR	20 (80)	1 (1–5)	5 (20)	NR	NR	NR	NR	NR	NR	NR
García-Noblejas 2014[29]	Bendamustine ± R	58	71 (43–90)	38 (67)	16 (28)	48 (87)	2 (1–6)	13 (21)	NR	15 (26) to most recent LoT	NR	39 (69.5)	NR	NR	9 (15), blastoid
Smith 2018[29]	Bendamustine ± R	20	68.6[f]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Karadurmus 2019[28]	Bendamustine ± R	18	65.6[d, f] (49–79)	15 (83.3)	7 (38.9)[b]	16 (88.9)[b]	1 (1–4)	5 (27.8)	NR	9 (50) to most recent LoT	NR	NR	NR	NR	NR
Visco 2021[9]	R-B	54	61 (35–70)[b]	42 (78)	NR	NR	1 (1–1)	22 (41)	NR	5 (9) to 1LT	22 (41) with 1LT	*High: 17 (32)*[d]	NR	NR	13 (25)[d]
Visco 2021[9]	R-BAC	76	55 (37–68)[b]	61 (80)	NR	NR	1 (1–1)	16 (22)	NR	10 (13) to 1LT	31 (41) with 1LT	*High: 26 (35)*[d]	NR	NR	17 (24)[d]
McCulloch 2020[13]	R-BAC	36	66 (43–81)	29 (80.6)	7 (20)	36 (100)[b]	2 (1–6)	15 (41.7)[g]	2 (5.6)	NR	16 (44.4) with 1LT	21 (80.8)	NR	NR	7 (19.4), blastoid
Other chemotherapy/immunochemotherapy-based approaches															
Smith 2018[29]	FC ± R	30	73.9[f]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Smith 2018[29]	CHOP ± R	37	72.8[f]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Smith 2018[29]	Chlorambucil ± R	19	83.9[f]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Smith 2018[29]	Cytarabine (DHAP, CHOP/DHAP, HyperCVAD) ± R	38	62.5[f]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Kroschinsky 2019[30]	DHAP	10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Lamm 2013[38]	R-ADOx	12	69 (57–87)[d]	12 (100)	2: 11 (91.7)[d]	12 (100)[d]	3 (1–9)	NR	NR	NR	NR	6 (50.0)[d]	NR	NR	1 (8.3), pleomorphic[d]

(*Continued*)

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Table 1 (Continued).

First author, publication date	Treatment	Patients, N	Age (years), *median (range)*	Male gender, n (%)	ECOG score ≥2, n (%)	Ann-Arbor stages III–IV, n (%)	No. of previous LoT, *median (range)*	Previous auto-SCT, n (%)	Previous allo-SCT, n (%)	Refractoriness to previous LoT, n (%)	POD24, n (%)	MIPI or sMIPI intermediate–high, n (%)	Ki-67 index ≥30%, n (%)	TP53 aberrations n (%)	Blastoid or pleomorphic histology, n (%)
Other strategies															
Skarbnik 2017[35]	Bortezomib	53	70.8[f]	37 (70)	NR	28 (97)	1[f]	5 (9)	NR	21 (40) to the most recent LoT	NR	NR	NR	NR	NR
Stefoni 2018[31]	Lenalidomide	70	67 (45–85)	50 (71.4)	20 (28.6)[d]	56 (80)[d]	2.5 (1–10)	36 (51.4)	NR	16 (22.8) to 1LT; 32 (45.7) to most recent LoT	NR	NR	NR	NR	NR
Zinzani 2015[32]	Lenalidomide	33	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hughes 2019[33]	Venetoclax	10	NR	NR	NR	10 (100)	NR	NR	0 (0)	NR	NR	NR	NR	NR	6 (60), blastoid
Iacoboni 2020[36]	CAR-T	33	67 (47–79)	29 (88)	≥1: 18 (55)	29 (88)[d]	2 (1–8)	12 (36)	5 (15)	7 (21) primary refractory disease	NR	23 (70)	16 (49)[d]	4 (12)[d], TP53	9 (27)
Romancik 2021[42]	CAR-T	52	66 (47–79)[d]	43 (82)	5 (10)[d]	IV: 41 (78)[b]	3 (2–8)	21 (40)	2 (4)	NR	26 (50) with 1LT	20 (68)[d]	30 (83)[d]	9 (39)[d], del(17p)	12 (30)[d]
Herbaux 2021[43]	CAR-T	47	67 (45–79)[h]	93.6%[c]	21.1%[c,d]	NR	3 (2–8)	34%[c]	NR	NR	NR	NR	78.60%[c,d]	NR	NR
Wang 2021[44]	CAR-T	93	67 (34–89)[d]	75 (81)	8 (9)[d]	81 (88)[d]	3 (1–9)	25 (27)	4 (4)	41 (44) to most recent LoT	NR	63 (88)[d]	66 (77)[d]	31 (46)[d]	38 (40.8)[d]

Notes: [a]Unless otherwise specified, the variables correspond to the time of relapse or treatment initiation, [b] at diagnosis; [c] n not reported; [d]unclear time of assessment; [e]line of therapy not reported; [f]range not reported; [g]in first-line therapy; [h]at registration in DESCAR-T French national registry.

Abbreviations: 1LT, first-line therapy; allo-SCT, allogenic stem cell transplantation; auto-SCT, autologous stem cell transplantation; BTK, Bruton's tyrosine kinase; CAR-T, chimeric antigen receptor T-cell therapy; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; DHAP, dexamethasone, cytarabine, and cisplatin; ECOG, Eastern Cooperative Oncology Group; FC, fludarabine and cyclophosphamide; hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; LoT, lines of therapy; MIPI, Mantle Cell Lymphoma International Prognostic Index; NR, not reported; POD24; progression of disease within 24 months; R, rituximab; R-ADOx, rituximab, Ara-C, dexamethasone, and oxaliplatin; R-B, rituximab and bendamustine; R-BAC, rituximab, bendamustine, and cytarabine; R/R MCL, relapsed/refractory mantle cell lymphoma; sMIPI, simplified Mantle Cell Lymphoma International Prognostic Index.

Table 2 Effectiveness Outcomes of Patients Treated with Bruton's Tyrosine Kinase (BTK) Inhibitors

First author and publication date	Treatment	PFS (months), *median*	OS (months), *median and/or % at the specified time*	ORR (%)	CR (%)	Follow-up (months), *median*
Ibrutinib as monotherapy						
Broccoli 2018[10]	Ibrutinib	12.9	16	36.4	18.2	38
Yi 2021[34]	Ibrutinib	20.8	79.1% at 2 years	64.8	NR	30.5
McCulloch 2021[13]	Ibrutinib	17.8	23.9	69	27	24
Tucker 2021[37]	Ibrutinib	12	18.5	NR	NR	60
Jeon 2019[21]	Ibrutinib	27.4	35.1	64	15	NR
Epperla 2017[14]	Ibrutinib	15	22	65	33	NR
Visco 2021[9]	Ibrutinib	24	Approx. 38	NR	NR	NR
Sancho 2022[22]	Ibrutinib	20	32	63.5	38.1	19.4
Sharman 2021[23]	Ibrutinib	19.6	25.8	NR	NR	16.1
Cencini 2021 [40]	Ibrutinib	17	34.8	62.3	39.1	15.6
Janssens 2021[40]	Ibrutinib	18.6	32.2	95.9	39.5	24.3
Obr 2021[41]	Ibrutinib	7.9	12.4	66	30	12.6
Zhang 2022[24]	Ibrutinib	18.5	28.2	41 (60.3)	11 (16.2)	20.5[a]
Ibrutinib in combination						
Zhang 2022[24]	Ibrutinib + Other agents	30.8	Not reached	45 (84.9)	23 (43.4)	20.5[a]
Fabbri 2020[39]	Ibrutinib + Venetoclax	NR	NR	100	50	NR

Notes: [a]For all patients in the study regardless of treatment.
Abbreviations: CR, complete response; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

The second study described the cases of four patients treated with ibrutinib and venetoclax, who were mostly males with a median age of 47 years at diagnosis.[39] All of them had previously been treated with rituximab, high dose cytarabine, and anthracycline and presented an ECOG score between 0 and 1, as well as an IV stage MCL. Three patients out of four presented with three high risk features: high MIPI value, Ki-67 index >30%, and blastoid or pleomorphic histology (Table 1).

The survival outcomes of both studies are also shown in Table 2. The treatment strategies reported in the first study resulted in a median PFS of 30.8 months, an ORR of 84.9%, and a CR of 43.4%, with a median follow-up of 20.5 months (for the overall study population).[24] Moreover, the efficacy of ibrutinib plus venetoclax in the second study was reported in terms of ORR and CR, which were 100% and 50%, respectively.[39]

Chemotherapy/Immunochemotherapy-Based Strategies

The strategies based on chemotherapy/immunochemotherapy included eight assessments of bendamustine regimens reported in seven studies;[9,13,25–29] one of fludarabine and cyclophosphamide (FC) regimens alone or combined with rituximab;[29] one of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) regimens alone or combined with rituximab;[29] one of chlorambucil regimens alone or combined with rituximab,[29] two of cytarabine-based regimens;[29,30] and one of rituximab, Ara-C, dexamethasone, and oxaliplatin (R-ADOx) regimens[38] (Table 1).

Bendamustine-Based Strategies

Seven studies assessed eight bendamustine-based approaches, five with bendamustine either with or without rituximab (bendamustine ± R);[25–29] one with bendamustine and rituximab (B–R);[9] and two with rituximab, bendamustine, and cytarabine (R-BAC).[9,13] These treatment regimens included between 18 and 76 patients (n = 8), predominantly males (between 67% and 83.3%, n = 5), with median ages at treatment initiation varying from 66 to 71 years (n = 3). The percentages of patients with an ECOG score ≥2 and a III–IV Ann-Arbor stage ranged from 20% to 38.9% (n = 3), and from 80% to 100% (n = 4), respectively. The patients had received a median of one to three previous lines of therapy (n = 6), and 20% to 41.7% of them, a previous auto-SCT (n = 6). As for response to previous lines of therapy, between 9% and 13% of the patients were

refractory to first-line therapy (n = 2), whereas 26% to 50% of them were refractory to the most recent therapy/chemotherapy (n = 2). In addition, 41% to 44.4% of the individuals were POD24 to their first-line therapy (n = 3). The proportion of patients presenting with an intermediate-high MIPI/sMIPI and a blastoid or pleomorphic MCL ranged from 69.5% to 80.8% (n = 2), and from 15% to 25% (n = 4), respectively (Table 1).

Patients treated with bendamustine-based approaches presented considerably variable survival outcomes, with a median PFS ranging from 10.1 to 25.9 months (n = 5) and a median OS from 12.5 to 43 months (n = 4). The ORR and CR ranged from 70% to 86% (n = 6), and from 40% to 61.1% (n = 5), respectively (Table 3). Median follow-ups ranged from 10 to 22 months.

Other Chemotherapy/Immunochemotherapy-Based Approaches

The general characteristics of the remaining chemotherapy/immunotherapy-based treatment regimens can also be seen in Table 1, whereas their corresponding outcomes are summarized in Table 3. The study of Smith et al included the assessments of FC, CHOP, chlorambucil, and cytarabine-based regimens.[29] The analysis of FC was performed on 30 patients with a median age at treatment onset of 73.9 years, resulting in a median OS of 9.6 months. Similarly, the assessment of CHOP included 37 patients with a median age at treatment initiation of 72.8 years; their median OS was 9.6 months as well. In contrast, the assessment on chlorambucil included a lower number of patients (n = 19), who were older than those described before (median of 83.9 years) and reported a shorter survival (median OS of 7.2 months). Moreover, the analysis of cytarabine-based regimens —DHAP, CHOP/DHAP, and HyperCVAD, alone or combined with rituximab— was performed among 38 younger patients (median age of 62.5 years) and yielded a median OS of 6.0 months. The other analysis of a cytarabine regimen (specifically, a modified DHAP regimen) included 10 patients and reported a 5-year PFS and OS of approximately 60%, along with an ORR of 50%, and a CR of 10%, with a median

Table 3 Effectiveness Outcomes of Patients Treated with Chemotherapy or Immunochemotherapy

First author and publication date	Treatment	PFS (months), *median and/or % at the specified time*	OS (months), *median and/or % at the specified time*	ORR (%)	CR (%)	Follow-up (months), *median*
Bendamustine-based approaches						
Rigacci 2012[25]	Bendamustine ± R	10% at 4 months	39% at 10 months	70	40	12/10[a]
Warsch 2012[26]	Bendamustine ± R	NR	Not reached	80	48	12
García-Noblejas 2014[27]	Bendamustine ± R	16	32.4	86	55	16
Smith 2018[29]	Bendamustine ± R	NR	12.0; 52.9% at 1 year	NR	NR	NR
Karadurmus 2019[28]	Bendamustine ± R	25.9	74.9% at 2 years	72.2	61.1	22
Visco 2021[9]	R-B	13	Approx. 43	NR	NR	NR
Visco 2021[9]	R-BAC	25	Approx. 38	73	NR	NR
McCulloch 2020[13]	R-BAC	10.1	12.5	83	60	18
Other chemotherapy/immunochemotherapy-based approaches						
Smith 2018[29]	FC ± R	NR	9.6; 44.7% at 1 year	NR	NR	NR
Smith 2018[29]	CHOP ± R	NR	9.6; 44.8% at 1 year	NR	NR	NR
Smith 2018[29]	Chlorambucil ± R	NR	7.2; 38.4% at 1 year	NR	NR	NR
Smith 2018[29]	Cytarabine (DHAP, CHOP/DHAP, HyperCVAD) ± R	NR	6.0; 31.7% at 1 year	NR	NR	NR
Kroschinsky 2019[30]	DHAP	Approx. 60% at 5 years	Approx. 60% at 5 years	50	10	64
Lamm 2013[38]	R-ADOx	9.3	Not reached	75	33.3	14.7

Notes: [a]For OS at 24 months and PFS at 20 months, respectively.
Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; CR, complete response; DHAP, dexamethasone, cytarabine, and cisplatin; FC, fludarabine and cyclophosphamide; hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R, rituximab; R-ADOx, rituximab, Ara-C, dexamethasone, and oxaliplatin; R-B, rituximab and bendamustine; R-BAC, rituximab, bendamustine, and cytarabine.

follow-up of 64 months.[30] As for the assessment of R-ADOx regimens, it included 12 male patients with a median age of 69 years and a median of 3 previous lines of therapy. Most patients (91.7%) had an ECOG=2 and, all of them, an III–IV stage MCL, whereas only one (8.3%) presented with an aggressive MCL histology (pleomorphic). The efficacy outcomes of this approach included a median PFS of 9.3 months, an ORR of 75%, and a CR of 33.3%, with a median follow-up of 14.7 months.[38]

Other MCL Treatments

Besides the treatment regimens based on BTK inhibitors and chemotherapy/immunotherapy, we found other R/R MCL treatment approaches, including treatment regimens based on bortezomib (n = 1),[35] lenalidomide (n = 2),[31,32] venetoclax (n = 1),[33] and CAR T-cell therapies (n = 4).[36,42–44]

Bortezomib

The study of the treatment regimen based on bortezomib included 53 patients (70% males) with a median age at treatment initiation of 70.8 years, with almost all of those with available data (97%) being at stages III–IV. Patients had received a median of 1 previous line of therapy, and 9% of them had undergone a previous auto-SCT. In addition, 40% of the patients were refractory to the most recent treatment (Table 1). This study reported a median PFS of 4.7 months and a median OS of 11.3 months, with a median follow-up of 5.3 months[35] (Table 4).

Lenalidomide

The study by Stefoni et al included 70 patients (71.4% males) with a median age of 67 years. Of them, 28.6% had an ECOG score ≥2, and 80% were at an III–IV stage. Patients had a median of 2.5 lines of therapy, and more than half of them (51.4%) had received an auto-SCT. In this regard, 22.8% and 45.7% of the patients were refractory to the first and the most recent line of therapy, respectively (Table 1). The authors reported a median PFS of 13.8 months, a median OS of 32.5 months, an ORR of 47.1%, and a CR of 31.4%[31] (Table 4). Similarly, the study of Zinzani et al, which included 33 patients with R/R MCL treated with lenalidomide, reported a PFS of 13.9 months, an ORR of 45.5% and a lower CR of 12.1%[32] (Table 4).

Venetoclax

The study on venetoclax included 10 patients, of which 90% had previously been treated with ibrutinib, 60% presented with blastoid histology, and all of them were at an III–IV stage (Table 1). Venetoclax treatment resulted in a median PFS and OS of 6 months[33] (Table 4).

Table 4 Effectiveness Outcomes of Patients Treated with Other Strategies

First author and publication date	Treatment	PFS (months), *median and/or % at the specified time*	OS (months), *median and/or % at the specified time*	ORR (%)	CR (%)	Follow-up (months), *median*
Skarbnik 2017[35]	Bortezomib	4.7	11.3	NR	NR	5.3
Stefoni 2018[31]	Lenalidomide	13.8	32.5	47.1	31.4	NR
Zinzani 2015[32]	Lenalidomide	13.9	NR	45.5	12.1	NR
Hughes 2019[33]	Venetoclax	6	6	NR	NR	NR
Iacoboni 2020[36]	CAR-T	50.8% at 1 year	61.4% at 1 year	91	79	10.1
Romancik 2021[42]	CAR-T	82.7% at 6 months	89.0% at 6 months	88	69	4.2
Herbaux 2021[43]	CAR-T	57.9% at 6 months	NR	88	61.9	3.3
Wang 2021[44]	CAR-T	80.6% at 3 months	82.1% at 6 months	86	64	3

Abbreviations: CAR-T, chimeric antigen receptor T-cell therapy; CR, complete response; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

CAR T-Cell Therapies

Four studies evaluated the use of CAR T-cell therapies with brexucabtagene on R/R MCL patients.[36,42–44] They included 33 to 93 patients, mostly males (81% to 93.6%, n = 4), with median ages between 66 and 67 years (n = 4). The proportion of patients with an ECOG score ≥2 ranged from 9% to 21.1% (n = 3), whereas the percentage of individuals at III–IV stages was 88% (n = 2). The number of previous lines of therapy varied from 2 to 3 (n = 4), the proportion of patients who had previously received an auto-SCT and an allo-SCT ranged from 27% to 40% (n = 4) and from 4% to 15% (n = 3), respectively, and patients previously treated with BTKis ranged 82% to 100% (n = 2). Regarding prognostic factors, 68% to 88% of the individuals had a MIPI/sMIPI intermediate-high (n = 3), whereas 49% to 83% of them presented with a Ki-67 index ≥30% (n = 4). Moreover, TP53 aberrations and blastoid or pleomorphic variants were found in 12% to 46% (n = 3) and in 27% to 45% (n = 3) of patients, respectively (Table 1).

Regarding efficacy outcomes, two studies reported 6-month PFS, ranging from 57.9% to 82.7%, whereas other studies reported similar rates at different time points (3-month PFS of 80.6%, n = 1, and 1-year PFS of 50.8%, n = 1). However, the 6-month OS was relatively homogeneous among the studies (82.1% to 89.4%, n = 3). The ORR and CR were also very similar, ranging from 86 to 91% (n = 4) and from 61.9 to 79% (n = 4), respectively. Median follow-ups were variable, ranging from 3 to 10.1 months (Table 4).

Risk of Bias Assessment

Table S1 summarizes the analysis of the risk of bias of the studies included in the systematic review. Of them, 26 (86.7%) clearly described eligibility criteria, 25 (83.3%) reported consecutive inclusion of participants, and only 8 (26.7%) stated complete inclusion of participants. In addition, 16 (53.3%) studies measured the lymphoma in a standard and reliable way for all the participants, but only 9 (30.0%) studies reported using valid methods to diagnose it. Demographics and clinical characteristics of R/R MCL patients were clearly reported for each treatment regimen in 23 (76.7%) and 15 (50.0%) of the studies, respectively, although the latter would increase if we considered the whole study population (ie, not only patients with R/R MCL) and/or all the treatment regimens of the studies. All studies (n = 30, 100.0%) clearly reported efficacy outcomes (ie, treatment response and survival) and demographic information of the sites or center where the studies were conducted. Finally, the statistics were clearly reported in 18 (60.0%) studies.

Discussion

In this systematic review of the characteristics and treatment outcomes of patients with R/R MCL in the clinical practice, we found that most treatment regimens were based on BTKis (specifically, ibrutinib) or chemotherapy/immunochemotherapy strategies (especially those including bendamustine). As expected, most patients were males with a median age ranging from 65 to 75 years at treatment initiation. Of the treatment regimens reporting each variable of interest, approximately two thirds of them included a percentage of patients with an ECOG score ≥2 between 10% and 30%, most of them had ≥80% of the patients with an III–IV stage disease, reflecting the reality of the treatment of the disease in the routine clinical practice. All of them reported a median number of previous lines of therapy of between 1 and 3. The percentage of patients who had previously received an auto-SCT was quite variable, but ranged from 10% to 40% in approximately two-thirds of the treatment regimens, depending on the age and status of the patients included in the study. More than half of the patients presented with an intermediate-high MIPI/sMIPI in treatment regimens reporting it. In addition, the percentages of patients with high-risk features, such as TP53 aberrations, and a Ki67 ≥30% were reported in a small number of studies and ranged from 0% to 46%, and from 33.3% to 100% of the patients, respectively, further reflecting high variability among patients included in these studies. Additionally, the percentage of patients with blastoid or pleomorphic histology was variable, ranging from 3.4% to 27–40% in CAR T-cell studies where, as expected, patients are in later lines and have more high-risk features.

Interestingly, only studies using ibrutinib and CAR T-cells (brexucabtagene autoleucel) reported similar efficacy outcomes in RWE studies[7–10,13,28–31,33,35,36,39–43] compared to clinical trials (CTs).[6,45] When comparing the values of each outcome of interest, the real-world treatment regimens using ibrutinib monotherapy[8–11,14,21–24,34,37,40,41] yielded a wide range of values for all the studied variables where those of the pooled analysis fell.[7] As for the treatment response,

the lowest values of ORR were reported with the lenalidomide regimens[25,26] and the cytarabine regimen.[29] Of note, the ibrutinib study by Broccoli et al[10] reported an ORR of 36%, but physicians in the study erroneously considered that ibrutinib induced transient lymphocytosis as PD and stopped treatment. The highest ORR values were the ibrutinib-based regimens reported by Janssens and Fabbri et al,[38,39] all four CAR T-cell treatment regimens,[36,42–44] and one study reporting bendamustine ± rituximab.[27] Interestingly, the ORR rates reported in the bendamustine RWE studies (ranging 70% to 86%) were generally lower than those reported in clinical trials.[45–50] Regarding CR rates, the highest CR values corresponded to the CAR T-cell treatment regimens,[36,42–44] whereas the lowest ones were those reported by the ibrutinib monotherapy regimens of Jeon et al,[21] Zhang et al,[24] and the already mentioned Broccoli et al,[10] the DHAP regimen,[30] and the lenalidomide regimen reported by Zinzani et al.[32] It is important to note that, among ibrutinib studies, there was a high variability between CR rates, ranging from 39.5% to 15%[28,39] which may be due not only to the baseline characteristics of the patients and the line of therapy in which ibrutinib was used, but also to the response criteria used in the study, which may vary significantly in this type of retrospective routine clinical practice studies. Moreover, it has been widely described in the successive follow-ups of ibrutinib clinical trials[51] that ORRs and CR rates improve over time, so these differences may also be due to short follow-up periods.

Regarding survival outcomes, the highest median PFS values were found with ibrutinib[21,24] and bendamustine (median PFS ranging from 10.1 to 25.9 months)[9,28] regimens. The ibrutinib-based treatment regimens resulted in a wide range of median PFS values, from 7.9 to 30.8 months.[8–11,14,21–24,34,37,39–41] The PFS reported in the pooled analysis,[6] with a median follow-up of 9.7 years, was 12.5 months in all the population (median of 2 prior lines) and 25.4 months in patients with one prior line of therapy. Thus, the number of prior lines of therapy the patient received before ibrutinib treatment should be considered in order to contextualize PFS results of the RWE studies. In the study by Obr et al, recently updated,[52] reporting a median PFS of 7.9 months, patients were heavily pretreated, with 72% of the patients with 2 or more previous lines of therapy. On the contrary, in the studies reporting data from patients treated with ibrutinib as second-line therapy,[8,9,13,34,40] where the best PFS results are expected, PFS ranged between 17 and 24 months, in line with the results of the pooled analysis, considering unselected RWE populations. Conversely, bortezomib[35] and venetoclax[33] treatment studies yielded the lowest PFS values, potentially because in these studies they were used as monotherapy in late lines of therapy and in elderly patients.

The highest median OS values were also found among ibrutinib[8–11,14,21–24,37,40,41] and bendamustine[9,13,27] treatment regimens. All of the other treatment regimens reporting OS yielded lower median OS values, and the lowest ones were those corresponding to cytarabine,[29] bortezomib,[35] and venetoclax[33] regimens, with the last two being lower than those reported in CTs.[49–51] CAR-T regimen studies did not report median PFS and OS due to their short follow-up, but the longest follow-up by Iacoboni et al[36] reported an estimated 50.8% PFS and 61.4% OS at 12 months, very promising results in this heavily pretreated high-risk patient population. In this context, it is important to note that multivariate analysis have shown several prognostic markers to have a deleterious effect in PFS and OS besides previous lines of therapy in the context of R/R MCL and need to be considered when comparing the efficacy results reported in the different studies:[6] ECOG, sMIPI, bulky disease, early progression of disease (POD24 status), and ultra-high-risk features, such as blastoid/pleomorphic histology and TP53 mutation.

When looking at clinical trials assessing the efficacy of the same agents retrieved in this review for treating R/R MCL patients,[7,45–50,53–63] we observed some similarities in the efficacy outcomes. Regarding response outcomes, as in the real-world studies, the highest ORRs were reported by clinical trials assessing bendamustine,[47,48] CAR T-cell therapy,[61] and ibrutinib combined with rituximab.[56] Conversely, the lowest ORRs were those found in clinical studies evaluating lenalidomide[63] and bortezomib.[59] Additionally, studies using CAR T-cell therapy[61] and bendamustine[47] reported the highest CR rates, whereas the lowest CR values were those reported in clinical trials using bortezomib[59] and lenalidomide.[63] Conversely, the highest median PFS values corresponded to ibrutinib-based therapies[6,53,55] and CAR T-cell therapies, considering that CAR T-cell therapies have been tried mostly in a post iBTK setting, whereas bortezomib,[59,60] together with lenalidomide,[63] yielded the lowest median PFS values.

It is important to note that bendamustine-based therapy results in higher CR rates than ibrutinib monotherapy in RWE and CTs, which does not translate into improved PFS/OS results, which could suggest that, besides attaining a CR, a well-established endpoint that prolongs PFS,[6] continuous treatment may play an important role in delaying progression of the disease in MCL. One proof of that is that rituximab maintenance after front line therapy has been shown to delay progression of the disease and improve PFS/OS[64–66] and has thus been established as standard of care.

In the retrospective study MANTLE FIRST, Visco et al[9] compared second line ibrutinib, R-BAC, R-bendamustine, and a variety of other treatments in young R/R MCL patients. The CR rates obtained with R-BAC and R-Benda were 63% and 43%, respectively, whereas ibrutinib yielded a lower CR rate of 38%, which did not translate into a better PFS for R-BAC (mPFS2: 25 m.) and R-bendamustine (mPFS2: 13 m.) in comparison with ibrutinib (mPFS2: 24 m.); conversely, ibrutinib resulted in a significantly longer PFS in POD24 patients compared to those attained with R-BAC and R-bendamustine (p=0.02) besides CR rates, reflecting that the attainment of deep responses, in the setting of targeted continuous therapies, may not be the only goal of therapy and may be achieved later in time without direct impact on PFS.

This study has some limitations, mainly associated with the quality of the data but also with the difficulty of comparing outcomes in R/R MCL due to the heterogeneity of the disease. First, the methods to assess the treatment outcomes were not always described accurately in the retrieved studies, which may entail a measurement bias and affect results. Secondly, not all the studies reported the same data on the characteristics of patients and the studied outcomes. In this regard, most studies did not report the Ki-67 index or the presence of TP53 aberrations, which are prognostic factors of MCL and, thus, may affect the efficacy of the treatment. Besides, many studies were basket studies and not only involved patients with R/R MCL and one treatment regimen, but also patients with other types of lymphomas, treatment-naïve MCL, and/or different treatment approaches. Given that not all these studies reported the variables by type of lymphoma, naïve or relapsed status, or treatment approach, we could not always retrieve the data corresponding to R/R MCL patients for a given treatment approach. Another limitation relies on the high variability of real-world data, making it difficult to compare the different treatment regimens among them and from those obtained in clinical trials. However, most of these limitations are inherent to real-world data, which are essential to complement those of clinical trials.

Conclusion

To our knowledge, this is the first systematic review of the real-world evidence on R/R MCL treatments. Baseline characteristics of patients included in the studies reflect the reality of R/R MCL in a real-world setting and the heterogeneity of the disease. A very important part of the studies retrieved were ibrutinib monotherapy studies, maybe due to the increasing importance in the last few years of real-world evidence and the need to confirm clinical trial results in a routine clinical practice setting with unselected patient populations. Furthermore, ibrutinib was a first-in-class BTK inhibitor and its first publication in 2013[67] raised interest in the medical community due to the unprecedented efficacy results reported for a targeted agent in monotherapy, its favorable tolerability profile, and its convenience compared to classic chemoimmunotherapy strategies, something that may have boosted interest in confirming those results in the routine clinical practice. Chemoimmunotherapy is still being widely used in the R/R setting, as evidenced by the broad range of studies considered in this review, but the use of other targeted therapies is very limited. Regarding efficacy outcomes, the best results obtained in RWE studies are those of ibrutinib, CAR T-cells and bendamustine-based regimens, the first two similar to those reported in clinical trials. Those results have led to expert/guidelines recommendations prioritizing BTKis at first relapse and CAR T-cells as the best option after BTKi relapse.[68–71] However, these results should be interpreted with caution since they are limited by the quality of the real-world data available and the difficulty of comparing outcomes in R/R MCL due to the heterogeneity of the disease.

Expert Opinion

Despite recent advances that have prolonged survival, R/R MCL is clinically and biologically heterogeneous and is still a therapeutic challenge, with high-risk and early relapsed patients remaining an unmet medical need. There is no standard treatment for R/R MCL, but considering patients' advanced age, tolerability profile, and convenient administration, BTKis should be positioned as second line of therapy,[6] and CAR T-cells should be the approach to BTKi-relapsed patients.[61] However, access to CAR T is not universal, and more therapies in this setting are still needed.

In this sense, there is a huge development in the MCL field with many chemo-free and combination regimens with established chemoimmunotherapy backbones being studied not only in the relapsed setting, but to improve front-line treatment options and to move forward to earlier lines targeted and cellular therapies. For instance, the randomized Phase 3 SHINE study, where

continuous ibrutinib or placebo was combined with rituximab-bendamustine followed by rituximab maintenance in front-line elderly MCL patients, showed a promising 80.6-month median PFS for the ibrutinib + BR arm, which is the longest PFS ever reported for this type of non-candidate to auto-SCT patients. The results of the TRIANGLE trial, evaluating the use of ibrutinib alone or in combination with auto-SCT in the front-line setting in candidates to auto-SCT[72] are also worth mentioning. These trial results seem to indicate that auto-SCT, currently the most efficacious standard of care for front-line transplant-eligible patients, is not superior to front-line ibrutinib monotherapy in young fit MCL patients. This is an unprecedented result leading us to hypothesize that auto-SCT may be replaced with the addition of ibrutinib to the induction therapy in these patients as front-line treatment, avoiding auto-SCT-associated morbidities and mortality. These results, along with other promising clinical trials including new targeted agents in combination with other therapies, may bring these therapies to the front-line setting and help improve patient outcomes since diagnosis (MANGROVE, NCT04002297; SYMPATICO, NCT03112174; OAsIs, NCT02558816; ENRICH, BOVEN, NCT03824483). Furthermore, these new therapeutic schemes could decrease toxicity compared to the standard chemotherapy mentioned in this study, being more convenient and tolerable, opening new venues towards improving both efficacy and tolerability in the newly diagnosed MCL population, often enriched in non-transplant eligible, elderly unfit patients.

Additionally, many new targeted agents will play an important role in the upcoming years (the non-covalent BTKis such as pirtobrutinib, anti-ROR1 conjugate zilovertamab, bispecific antiCD20-CD3 antibodies epcoritamab, glofitamab, other CAR T-cells like lisocabtagene and other small molecules) and will eventually evolve to be used in the front-line setting, where therapies have been proved more efficacious. Conversely, it can be hypothesized that chemoimmunotherapy will have a small role, while targeted agents along with CAR-T cells will have a major role in the front-line setting and the first relapse, where the best results will be achieved, and the life of MCL patients will be prolonged. How these therapies will be incorporated to the daily clinical practice will depend on many factors, including access to novel therapies in different geographical regions. Irrespective of access, these agents and their combinations will have to be added to the treatment strategies according to patients' status and age. Furthermore, optimal sequencing will have to be determined, and those results will have to be confirmed in non-selected MCL populations, which are usually elderly and have comorbidities and use concomitant medications that may affect the outcomes of these treatments. The rise of RWE studies has been very positive to the medical community, expanding knowledge about these therapies, not only in terms of effectiveness, but also of their long-term security profile in real-world populations.

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The Characteristics of Compound Heterozygosity for Hemoglobin G-Makassar with Hb E in Malaysia

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CASE SERIES

The Characteristics of Compound Heterozygosity for Hemoglobin G-Makassar with Hb E in Malaysia

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Video abstract

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Background: Human hemoglobin of G-Makassar and hemoglobin E (Hb E) are hemoglobin variants that affect Beta (β) globin. Hb G-Makassar is a very rare variant while Hb E is estimated to affect at least one million people worldwide. Both Hb G-Makassar and Hb E can be inherited in the heterozygous, homozygous or compound heterozygous state. This case series describes the characteristics of four individuals with compound heterozygosity for Hb G-Makassar/Hb E cases in Malaysia. To the best of our knowledge, these are the only four individuals with this genotype reported in the literature.

Case Series: We present four cases of compound heterozygosity for Hb G-Makassar/Hb E identified from October 2014 to January 2021. All the cases were incidental findings whereby the screening Hb analysis showed the presence of peaks in both Hb S and Hb E zones on capillary electrophoresis (CE) and cation-exchange high-performance liquid chromatography (HPLC). Molecular analysis confirmed the findings of compound heterozygous Hb G-Makassar/Hb E. Two cases had a history of anemia secondary to unrelated conditions that resolved with treatment of the underlying cause. The other two cases were asymptomatic individuals who were detected through Malaysia's National Thalassemia Screening program. On the last follow-up, all the individuals were well, non-transfusion dependent, and had no reported history of chronic anemia, bleeding, hemolysis or thromboembolism complications.

Conclusion: The cases reported here highlight the possibilities for rare compound heterozygous states in multi-ethnicity populations such as Malaysia. Compound heterozygous Hb G-Makassar/Hb E individuals are clinically silent with laboratory values suggesting microcytic and hypochromic red blood cells. Further local epidemiology or population studies with genotyping tests are required for a better understanding of the diversity of its clinical phenotype.

Keywords: beta-thalassemia, compound heterozygous, Hb E, Hb G-Makassar, hemoglobinopathy

Introduction

Thalassemia and hemoglobinopathies are the most prevalent genetic disorders and one of the major public health problems in Malaysia. About 4.5% of Malaysian populations are heterozygous carriers for beta (β)-thalassemia.[1] Beta-thalassemia major is the most significant thalassemia with severe anemia requiring life-long blood transfusions for survival.[2,3] Among β-thalassemia, Hb E (β26; Glu-Lys) is the second most common abnormal structural variant of hemoglobin in the world after sickle cell hemoglobin (Hb S) and the most common variant in Southeast Asia.[4,5] Hb E is prevalent in Malaysia, accounts for 76.0% of the β-thalassemia mutations,[6] whereas Hb G-Makassar (β6; Glu-Ala) is very rare. Hb G-Makassar affects the same codon as Hb S (β6; Glu-Val), thus indistinguishable from Hb S by routine Hb analysis methods such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), or isoelectric focusing (IEF).[7,8]

Case Presentation

We describe a total of four individuals with compound heterozygous Hb G-Makassar/Hb E diagnosis from the various states of Malaysia.

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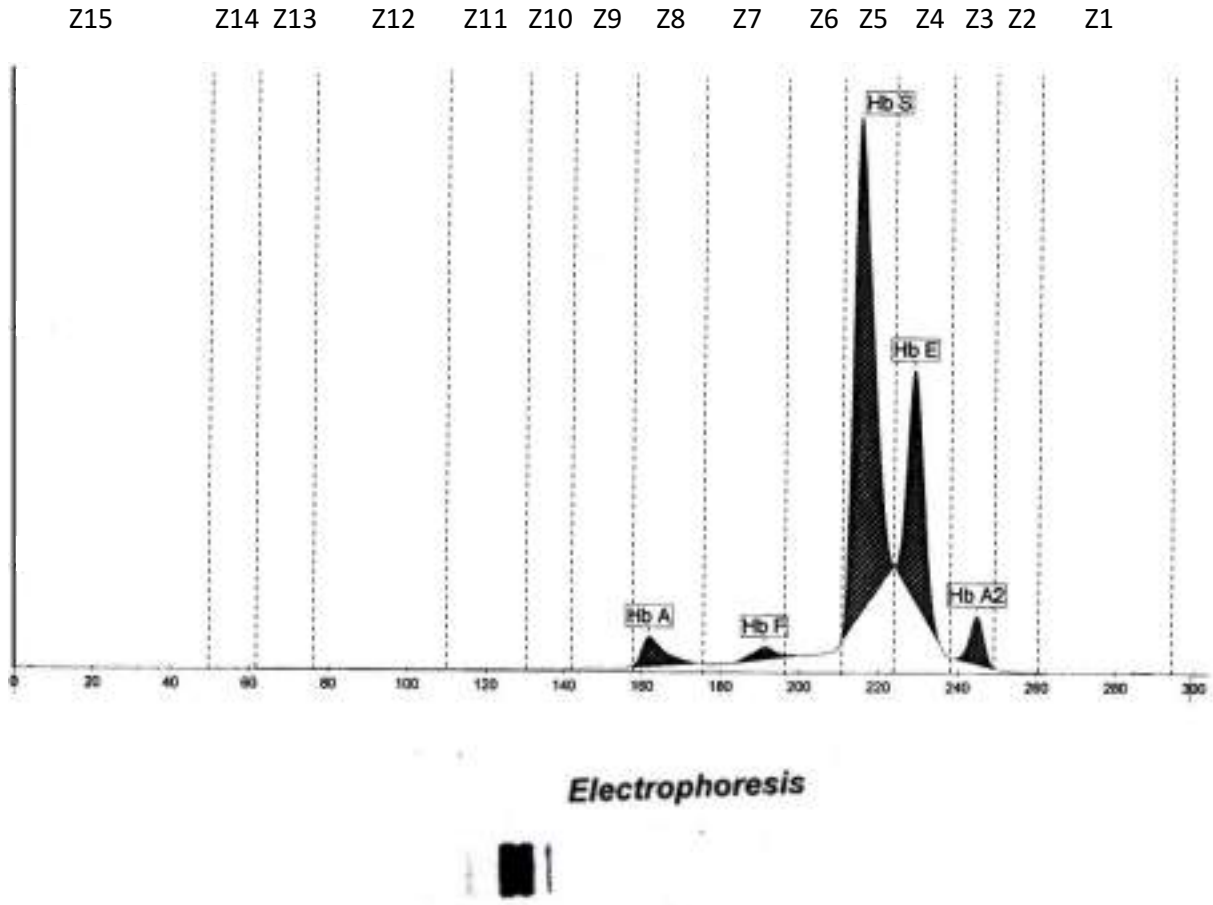
Case 1

The first case was a 45-year-old male with a history of lower gastrointestinal bleeding secondary to haemorrhoid. This case was reported by Mohamad et al.[7] He presented with anemic symptoms and complicated with iron deficiency anemia as a result of frequent bleeding haemorrhoids that required blood transfusions. His initial hematological parameters showed severe anemia with hypochromic microcytic red blood cells (RBCs) with Hb 5.7g/dl, mean corpuscular volume (MCV) 68.9fl and mean corpuscular hemoglobin (MCH) 20.2pg. During hospitalization, an incidental finding of a compound heterozygous Hb S/Hb E was suggested from CE (Figure 1). However, the genotyping of the β-globin gene had identified a compound heterozygous Hb G-Makassar/Hb E. His anemic symptoms improved after receiving iron therapy and haemorrhoidectomy. The blood count parameter during the Hb analysis is shown in Table 1. On follow-up evaluation at 57 years of age, he remained clinically well with no bleeding recurrence since his haemorrhoidectomy with normal hemoglobin concentration of 13.9g/dL and normal serum iron and ferritin level.



Fractions	%	Ref.%	Ref. g/dl
Hb A	4.6		
Hb F	2.1		
Hb S	63.0		
Hb E	25.7		
Hb A2	4.6		

Figure 1 Hb-electrophoresis chromatogram of Case 1 shows the presence of the Hb variant suggestive of Hb S and Hb E with their percentage.

Table 1 Demographic, Haematological Parameters, Iron Level and Alpha Mutation Study in All Cases with Compound Heterozygous Hb G-Makassar/Hb E in Malaysia

Subjects number (Case)	Age, year	Sex	State	Hb (g/dL)	RBC (10^{12}/L)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RDW-SD (fl), RDW-CV (%)	Iron level (umol/L)	Alpha (α)-globin DNA analysis
1	45	Male	Selangor	13.9	5.18	38.3	73.9	26.8	36.3	47.3, 17.5	2.4	NMD
2	1	Male	Putrajaya	10.9	5.20	-	61.0	20.6	33.8	33.2, 15.6	9.6	NMD
3	16	Female	Negeri Sembilan	12.3	5.54	35.9	64.8	22.2	34.3	45.9, 12.6	Not done	NMD
4	16	Male	Terengganu	15.4	6.08	43.7	71.9	25.3	35.2	38.8, 14.7	Not done	NMD

Notes: Normal ranges for adults: Hb - male 13.5–17.4g/dl, female 11.6–15.1g/dl; Hct – male 40.1–50.6%, female 35.1–44.9%; RBC - male 4.53–5.95 x 10^{12}/L, female 3.87–5.21 x 10^{12}/L; MCV 80.6–95.5fl; MCH 26.9–32.3pg; MCHC 31.9–35.5g/dL; RDW-SD 37.5–48.1fl; RDW-CV 12.0–14.8%. Normal ranges for paediatrics aged 6 months to 2 years: Hb 11.1–14.1g/dl; Hct 30.0–40.0%; RBC 3.9–5.1 x 10^{12}/L; MCV 75.0–87.0fl; MCH – 24.0–30.0pg; MCHC 31.0–37.0g/dL. Bold values are outside the normal range. Iron < 7 umol/L is iron deficient.
Abbreviations: Hb, hemoglobin; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean corpuscular volume; NMD, no mutation detected; RBC, red blood cell count; RDW, red cell distribution width; SD, standard deviation.

Case 2

The second case was a 12-month-old boy with underlying congenital hypothyroidism who was found to have a borderline low Hb during admission for fever. He had mild microcytic hypochromic anemia with a normal serum iron level (Table 1). The Hb analysis for both CE and HPLC were suggestive of compound heterozygous Hb S/Hb E, nevertheless the β-globin deoxyribonucleic acid (DNA) analysis confirmed compound heterozygous Hb G-Makassar/Hb E. He was euthyroid after treatment with L-thyroxine for three years. At the latest follow-up at the age of 6 years in 2019, he was well with Hb 12.6g/dl, RBC 5.5 × 10^{12}/L, MCV 68.1fl, MCH 22.8pg, MCHC 33.5g/dL and RDW 14.8%. He has normal development for his age and no longer on the follow-up during the phone interview in early 2022. He also has a family history of hemoglobin variants whereby the father and the brother are heterozygous Hb E. Hb analysis using the automated HPLC revealed raised Hb A2 that is suggestive of Hb E (Figure 2A and B). His mother is heterozygous Hb G-Makassar. Automated HPLC showed a peak at S-window suggestive of Hb S, but DNA analysis confirmed the diagnosis of heterozygous Hb G-Makassar (Figure 2C). Unfortunately, the HPLC chromatogram for Case 2 is not available.

Case 3 and Case 4

The third and fourth cases were a 16-year-old female and a 16-year-old male, respectively. They were noted to have microcytic hypochromic features although the Hb was within the normal range during Malaysia's National Thalassemia Screening program. Hb analysis screening for both CE and HPLC were suggestive of compound heterozygous Hb S/Hb E. However, the β-globin DNA analysis identified compound heterozygous Hb G-Makassar/Hb E. Further history in year 2022 at the age of 20 and 19 years old, respectively, revealed they were asymptomatic and denied any symptoms of anemia, history of transfusion-dependence, or features of hemolysis or thrombosis. The third case had normal renal and liver function tests while these tests were not done for the fourth case.

At the time of the study, all the individuals with compound heterozygous Hb G-Makassar/Hb E were alive, asymptomatic, and well. None has any complications such as chronic anemia, hemolysis, transfusion-dependency, thromboembolism, bleeding, severe infections, or hematological malignancy.

Laboratory Investigations

The definitive diagnosis for all the cases was done by direct DNA sequencing analysis of the *HBB* gene using ABI 3730XL DNA Analyser (Applied Biosystems, Foster City, CA, USA) to detect β-globin variants including Hb G-Makassar and Hb S simultaneously. The laboratory characteristics of compound heterozygous Hb G-Makassar/Hb E individuals are detailed in Table 1. DNA analysis for Alpha (α) mutation was performed by multiplex Amplification Refractory Mutation System polymerase chain reaction (ARMS-PCR) and multiplex gap-PCR to detect the common

non-deletional and deletional α-gene respectively. The test was performed in view of lower value of presumptive Hb E fraction, low MCV (median 68.4fl, range 61fl - 73.9fl) and MCH (median 23.8pg, range 20.6pg–26.8pg) for all the four individuals but did not reveal any mutations. Among them, only MCV and MCH showed persistently lower than reference ranges. Case 1 had a normal Hb (improved after the treatment given) and a low iron level at screening. He was then asymptomatic with normal blood parameters after undergoing surgical repair for hemorrhoids and correction of

A

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.99	1,748
F	0.5	---	1.09	14,655
Unknown	---	0.9	1.24	26,166
P2	---	3.2	1.37	91,046
P3	---	5.4	1.85	155,357
Ao	---	59.7	2.45	1,710,573
A2	27.2*	---	3.72	864,124

Total Area: 2,863,669

F Concentration = 0.5 %
A2 Concentration = 27.2* %

*Values outside of expected ranges

Analysis comments:

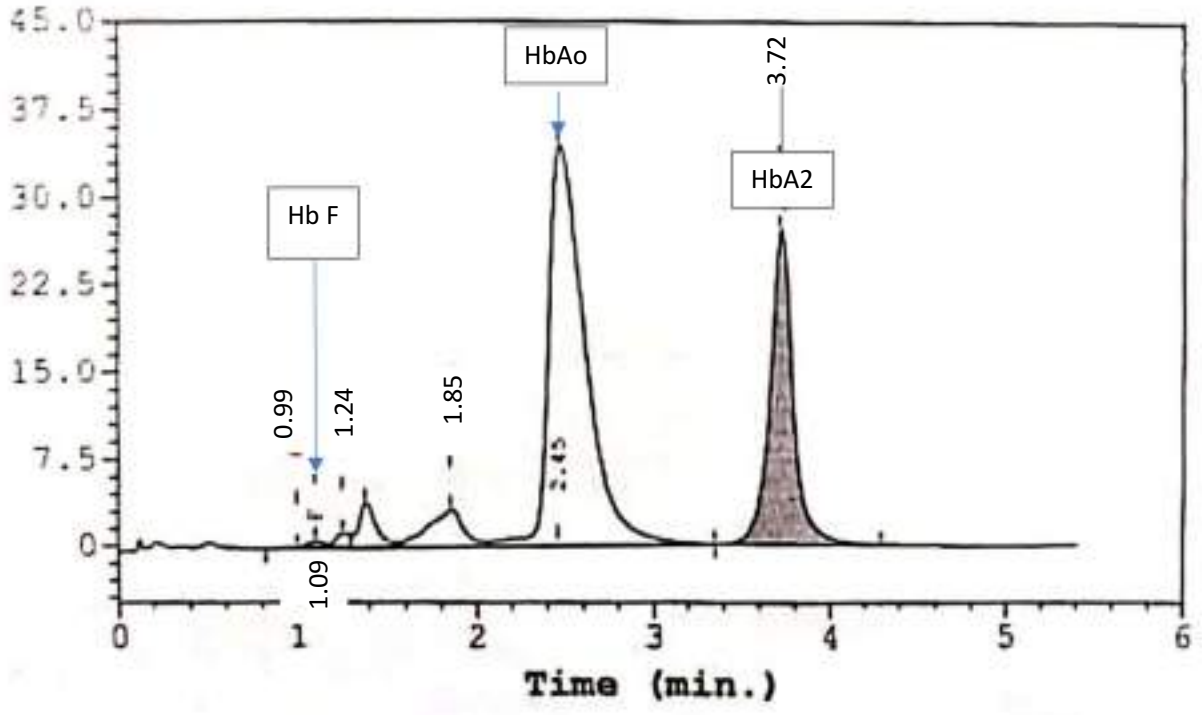


Figure 2 Continued.

B

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.99	1,093
F	0.7	---	1.10	15,051
Unknown	---	0.9	1.25	19,444
P2	---	2.7	1.37	60,185
P3	---	4.5	1.85	100,739
Ao	---	60.2	2.48	1,358,267
A2	28.0*	---	3.72	700,904

Total Area : 2,255,684

F Concentration = 0.7 %
A2 Concentration = 28.0* %

*Values outside of expected ranges

Analysis comments:

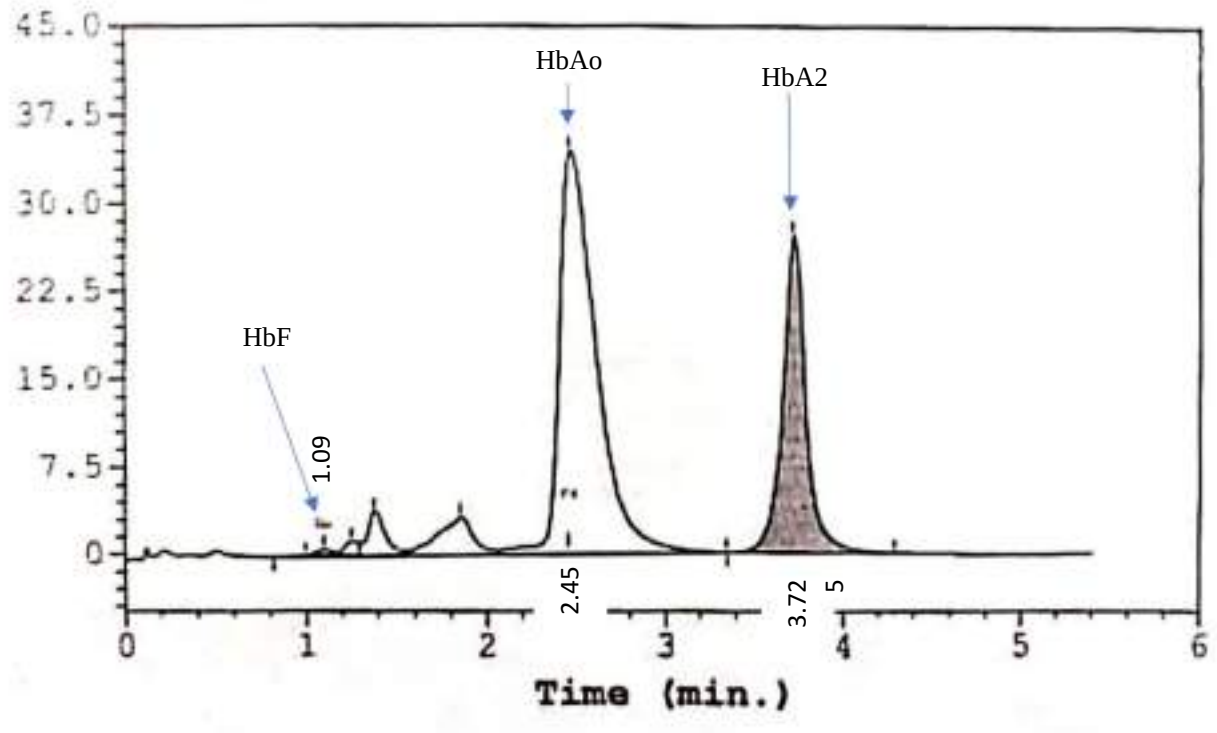


Figure 2 Continued.

C

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	1.01	646
F	0.2	---	1.10	4,853
Unknown	---	0.5	1.26	11,032
P2	---	2.4	1.37	49,112
P3	---	2.3	1.77	48,015
Unknown	---	0.6	2.19	12,640
Ao	---	47.6	2.51	978,701
A2	3.6*	---	3.68	81,121
S-window	---	42.3	4.41	868,225

Total Area : 2,054,345

F Concentration = 0.2 %
A2 Concentration = 3.6* %

*Values outside of expected ranges

Analysis comments:

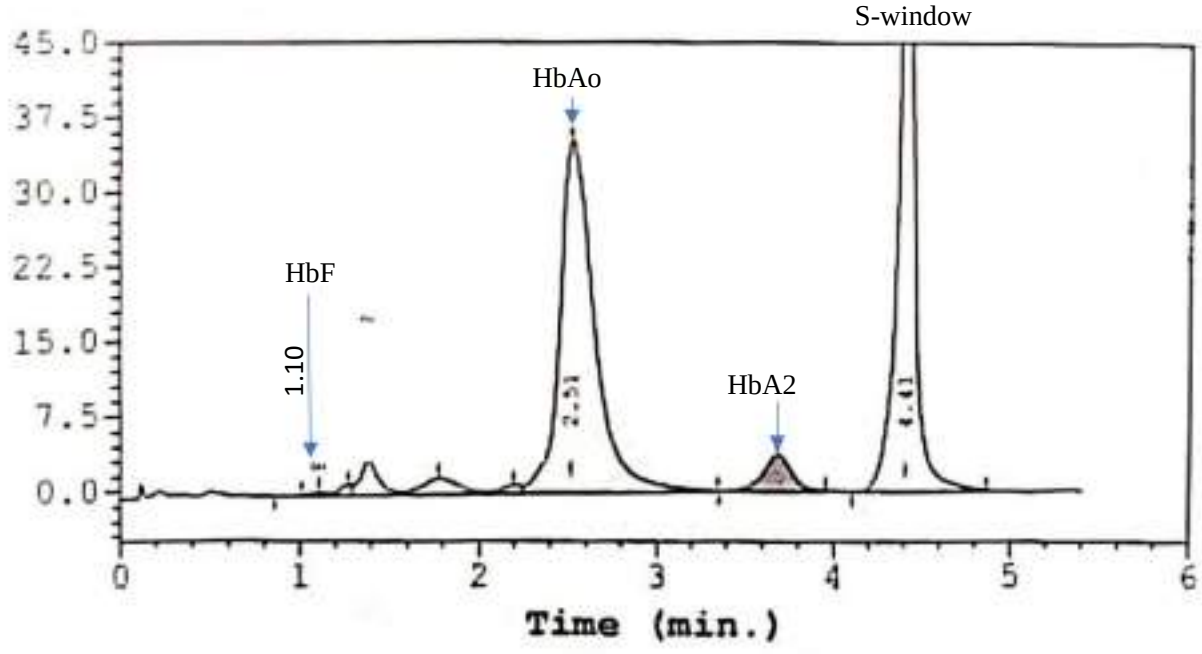


Figure 2 High-performance liquid chromatography (HPLC) analysis of family members of Case 2. (**A**) Hb E heterozygous father; (**B**) Hb E heterozygous brother; (**C**) Hb G-Makassar heterozygous mother.

iron deficiencies (except MCV persistently < 80fl). All of them showed microcytic hypochromic red cells but absent of sickle cells or features of hemolysis on the peripheral blood smear that would be seen in people with Hb S. We noted that the RBC was normal in Cases 1 and 2 but high in Cases 3 and 4. We postulated that Case 1 and Case 2 had iron deficiency anemia on presentation while Case 3 and Case 4 were normal leading to this discrepancy. Reticulocytes and total bilirubin were not raised in Case 1, 2 and 3 but not tested in Case 4.

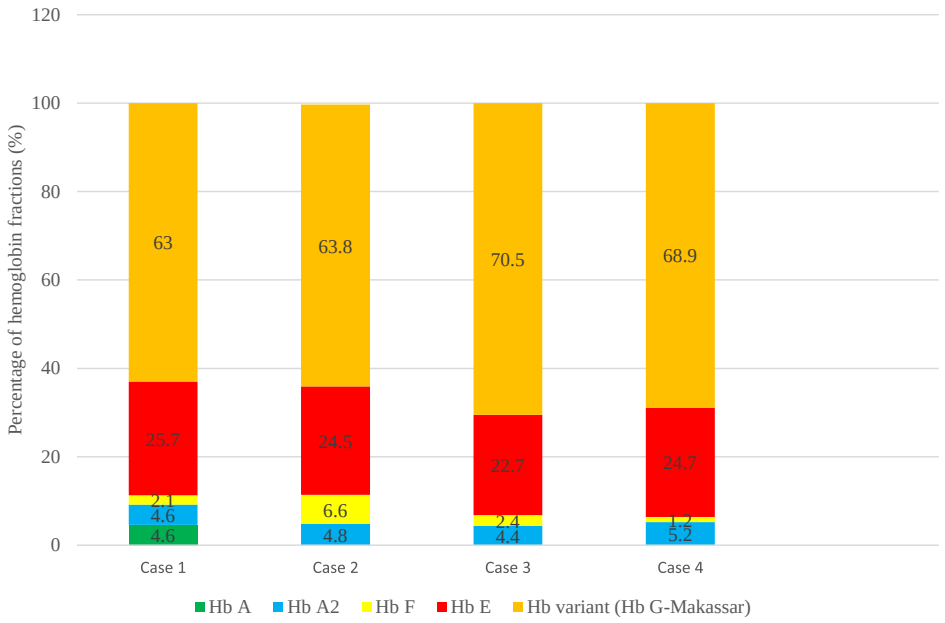


Figure 3 Hemoglobin fractions by capillary electrophoresis of four individuals with compound heterozygous Hb G-Makassar/Hb E. We assumed the Hb A (4.6%) level in case 1 was from the donor as he received a transfusion prior to the screening.

As shown in Figure 3, Hb G-Makassar level was high within this cohort with a mean of 66.6% ± 3.7. The Hb E level ranged from 22.7% to 25.7% of the total hemoglobin with a mean of 24.4% ± 1.2 and 27.0% ± 1.9 on CE and HPLC, respectively. We assumed the Hb A (4.6%) level in case 1 was from the donor as he received transfusion prior to the screening. There was no concomitant α-thalassemia in these individuals.

Discussion

Hb G-Makassar is a nonpathological β-chain variant characterized by a single nucleotide substitution GAG-> GCG at the β6 or A3 position that changes normal glutamyl residue to alanyl residue (β6 Glu→Ala). This rare mutation was first identified in Makassar, Sulawesi (Celebes), the Republic of Indonesia in 1969.[9] Hb G-Makassar heterozygotes and homozygotes are asymptomatic with normal complete blood count parameters though its structural change occurs at the same position as that in Hb S.[10] Furthermore, Hb G-Makassar appears to have the properties of normal Hb A and does not polymerize or lead to hemolysis like sickle cells.[11,12] In contrast, the Hb G-Makassar/β^0-thalassemia compound heterozygote has hematologic features of thalassemia trait.[8]

Hb E gene is a mutant form of the β-globin gene that encodes lysine instead of glutamate at position 26. This β-E chain is inefficiently produced because of a novel cryptic messenger RNA splicer site, leading to thalassemic RBC indices such as microcytosis.[13,14] Individuals with homozygous Hb E (Hb EE) and Hb E trait are clinically normal but compound heterozygous Hb E/β^+ or Hb E/β^0 thalassemia may have clinical manifestations of thalassemia, including compound heterozygosity for Hb E and Hb S (Hb SE).[15–18] Hb SE may result in sickle cell crises such as vaso-occlusive pain crises and hemolytic anemia, particularly during exposure to stress conditions or deoxygenated states such as infection or hypoxemia, unlike compound heterozygous Hb G-Makassar/Hb E, which is essentially asymptomatic. It is important to distinguish Hb E disorders diagnostically as it has different clinical courses amongst different genotypes. For example, Hb E beta-thalassemia (Hb E/β) has a wide phenotype that ranges from mild anemia to severe transfusion-dependent thalassemia major.[19] Compound heterozygous Hb G-Makassar/Hb E in our cohort can be considered mild phenotype of β-thalassemia as they are clinically normal though having some thalassemia trait red cell indices.

In Malaysia, Hb E/β-thalassemia (34.4%) is the most common form of β-thalassemia and majority in Malay ethnicity, followed by β-thalassemia major (33.5%), hemoglobin H (Hb H) disease (18.3%), β-thalassemia intermedia (9.4%), and 'others' (4.5%). The 'others' group includes other forms of Hb H disease, Hb Lepore Hollandia, α-thalassemia syndrome,

δβ-thalassemia, and other thalassemia disorders requiring regular blood transfusions.[20] However, the prevalence and incidence of Hb G-Makassar including compound heterozygous Hb G-Makassar/Hb E remain less elucidative. The incidence of both Hb E and Hb G-Makassar could be underrepresented as they are usually asymptomatic and red cell indices are commonly unremarkable on a routine blood counts check-up, hence are not likely to be sent for screening. Nevertheless, Hb E can be easily detected on Hb electrophoresis screening but the incidence of Hb G-Makassar may be underestimated as DNA molecular analysis is required for diagnostic confirmation. Therefore, in asymptomatic individuals with borderline or lowish MCV/MCH levels who are suspected to have Hb E and Hb S peaks on HPLC or CE, we suggest they also undergo genotyping.

Some genetic modifiers and prognostic indicators affect the severity of phenotype, including type of β-chain mutation, Hb F levels, concomitance of α-thalassemia, age of presentation and splenomegaly. Thromboembolism risk is high in post-splenectomised thalassemia patients due to a hypercoagulable state.[21] Morbidity from iron overload in non-transfused patients is also commonly seen, secondary to increased gastrointestinal iron absorption.[22] Hb E/β and Hb SE patients require regular monitoring because of risk of cardiopulmonary disease such as pulmonary hypertension and cardiac failure secondary to iron overload, chronic thromboembolism, and hemolysis-induced nitric oxide deficiency.[14,23–25] The laboratory characteristics and the phenotypic variability in compound heterozygous Hb G-Makassar/Hb E individuals are not widely reported. As opposed to Hb Eβ and HbE/S, all our cases of compound heterozygous Hb G-Makassar/Hb E were well with no clinical symptoms and did not show any complications during individual follow-up. None were on any treatment or needs regular follow-up. Our cases clearly show that compound heterozygous Hb G-Makassar/Hb E is a mild phenotype of hemoglobinopathy and asymptomatic.

For the laboratory tests, our compound heterozygous Hb G-Makassar/Hb E individuals showed unremarkable haematological parameters except for low MCV and MCH. The low MCV < 74fl, MCH < 24pg and lower Hb E < 25–26% are likely suggestive of Hb E concomitant α-thalassemia trait[26,27] and Hb E level <24% was reported superior to MCV and MCH for differentiating the heterozygous Hb E with or without α^0-thalassemia trait.[28] Interestingly, none of our cases had α mutations. Hb E percentage also can be reduced by iron deficiency. However, we could not conclude coexisting iron deficiency as not all individuals did iron studies. The high level of Hb G-Makassar (>60%) could explain the mild phenotype in our cohort as there is preferential binding of α-globin chain subunit to Hb G-Makassar than Hb E (range 22.7% to 25.7%). The Hb E level on CE was lower than HPLC and is consistent with the study by Hafiza et al. This is due to the Hb E and HbA2 coeluted at the same retention time on HPLC meanwhile CE measured the actual level of Hb E in the sample.[29] On alkaline Hb electrophoresis, Hb E migrates with C, O Arab and A2 whilst in acid pH electrophoresis testing, it migrates with Hb A2.

Hb G-Makassar has been reported as a promising new treatment target that may assist in gene editing therapy in sickle cell disease patients based on installing the Hb G-Makassar variant to replace the pathogenic Hb S allele.[30–32] The potential for this approach to be used to treat sickle cell disease (SCD) in humans is an area of active investigation. Recently, non-clinical studies in homozygous Hb S cells showed that gene editing of the pathogenic Hb S allele into Hb G-Makassar with an adenine base editor led to normal hemoglobin function in vitro and rescued mice with sickle cell disease in vivo.

Therefore we suggest a local epidemiology or population studies with genotyping tests to elucidate the status of Hb G-Makassar in the future for a better understanding of the diversity of its clinical phenotype.

Conclusion

The cases reported here highlight the possibilities for rare compound heterozygous states in multi-ethnicity populations such as Malaysia. Individuals with compound heterozygosity for Hb G-Makassar and Hb E had laboratory values demonstrating microcytic and hypochromic RBCs but were clinically asymptomatic without evidence of chronic anemia, hemolysis, bleeding or thrombosis. These findings may provide a valuable reference for prenatal diagnosis and genetic counseling as well as data to support sickle cell disease therapy based on installing the Hb G-Makassar variant to replace the pathogenic Hb S allele.

Abbreviations

Hb, Hemoglobin; CE, Capillary electrophoresis; HPLC, Cation-exchange high-performance liquid chromatography; IEF, Isoelectric focusing; RBCs, Red blood cells; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean cell hemoglobin concentration; DNA, Deoxyribonucleic acid; RDW, Red cell distribution width.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Declaration of Parental Consent

Ethical approval to report this case series was obtained from Medical Research and Ethics Committee, (NMRR-21-1408-60675, IIR), National Institutes of Health, Ministry of Health Malaysia, Malaysia. The authors certify that they have obtained all appropriate patient consent forms. In the form the parental(s) has/have given his/her/their consent for his/her/their child images and other clinical information to be reported in the journal. The parents understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed. The case 1 patient in this study has given written consent to participate as well as consent to publish his data.

Consent for Publication

This study was conducted in accordance with the fundamental principles of the Declaration of Helsinki.

Acknowledgments

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Disclosure

Guo Chen is an employee and stockholder of Beam Therapeutics Inc. The authors report no other conflicts of interest in this work.

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Identification of Novel Hb Guiyang [HBA2: c.151C > A α2 50 (CE8) His- Asn] and Phenotype- Genotype Correlation of Abnormal Hemoglobins in Guizhou, Southwest China

Ya-Ping Chen, Heng Wang, Lei Wang, Dan Xie, Min Guo, Jiang-Fen Wu, Bang-Quan An & Sheng-Wen Huang

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ORIGINAL RESEARCH

Identification of Novel Hb Guiyang [HBA2: c.151C > A α2 50 (CE8) His- Asn] and Phenotype- Genotype Correlation of Abnormal Hemoglobins in Guizhou, Southwest China

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Purpose: To analyze the composition of abnormal hemoglobin and the relationship between genotype and phenotype by screening abnormal hemoglobin in a subpopulation of Guizhou, China.

Patients and Methods: Routine blood evaluation, capillary electrophoresis of hemoglobin, and mutation of α - and β - thalassemia genes were evaluated in 19,976 individuals for thalassemia screening in Guizhou. Sanger sequencing of HBA1, HBA2 and HBB genes was performed in samples with abnormal bands or unexplained increases of normal bands. The types of abnormal hemoglobin were obtained by sequence analysis.

Results: Abnormal hemoglobin was detected in 84 individuals (detection rate, 0.42%). Ten types each of α and β globin chain variants were detected, including most commonly Hb E, Hb New York and Hb Port Phillip. In this study, the abnormal Hb Mizuho was identified for the first time in a Chinese population, and a novel abnormal hemoglobin Hb Guiyang (HBA2: c.151C > A) was detected for the first time. Except for Hb Mizuho, other abnormal hemoglobin heterozygotes without thalassemia or iron deficiency had no significant hematological changes.

Conclusion: This study enriched the molecular epidemiological data of abnormal hemoglobin in Guizhou, China and provided reference data for genetic counseling and prenatal diagnosis of abnormal hemoglobin.

Keywords: abnormal hemoglobin, routine blood examination, capillary electrophoresis, DNA sequencing, Hb Mizuho, *HBA2*

Introduction

Hemoglobinopathy is a type of autosomal monogenic genetic disease with a globin gene mutation that results in insufficient hemoglobin synthesis or structural abnormalities, which is widely distributed worldwide, especially in the Mediterranean, Africa and Southeast Asia.[1] Approximately 7% of the world's population are hemoglobinopathy carriers, and 330,000 newborns are born each year with hemoglobinopathy (91%, sickle cell disease), which causes 3.4% of deaths in children under the age of 5.[2] Clearly, this disorder poses a major worldwide public health problem.

Hemoglobinopathies are divided into two major categories, one is abnormal hemoglobin caused by mutations in the globin gene resulting in abnormal structure of the globin chain, also known as hemoglobin variant, an example of which is sickle cell disease. The other is inhibition of globin peptide chain synthesis caused by defects or mutations in the globin gene, resulting in chronic hemolytic anemia called thalassemia.[3] According to the human hemoglobin variants and thalassemia mutation database HbVar (https://globin.bx.psu.edu/hbvar/), 1429 hemoglobin variants have

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been identified, and this number is still rising. Epidemiological data from various localities have shown that there are ethnic and geographical differences in the incidence of hemoglobinopathy and the distribution of mutation types.[4] In most hemoglobinopathy-endemic populations, α- and β-thalassemia coexist with various abnormal hemoglobins.[5] Abnormal hemoglobin causes symptoms in individuals ranging from almost asymptomatic to severe hemolytic anemia and the hemoglobin molecule is characterized by structural abnormalities due to changes in the conformation of the globin peptide chain. Although the mutation types include base deletion and insertion, frameshift mutation, stop code mutation, fusion genes resulting from exchange between different genes, the vast majority are single amino acid substitutions caused by point mutations. Although most abnormal hemoglobins have limited clinical significance, a small number of homozygotes or if combined with thalassemia will present with significant anemia, such as Hb S/S and Hb E/β^0.[6,7]

The study of hemoglobinopathy in China was initiated in the 1960s, and in the 1980s, Chinese scientists conducted a general survey of hemoglobinopathy in nearly one million people covering 28 provinces nationwide. That survey showed that the average carrier rate of abnormal hemoglobin in Chinese population was 0.33%, and the distribution showed a significant difference between the south (0.37%) and the north (0.29%).[8] Although genotype identification was not performed then due to technical reasons, with the rapid development of sequencing technology, abnormal hemoglobin genotypes have been continuously identified throughout China. Because the carrier rate of abnormal hemoglobin is much lower than thalassemia, and often asymptomatic, only a few abnormal hemoglobins have been identified when the indication was thalassemia screening and gene sequencing prompted by thalassemia-like clinical manifestations. Southwest China is known as a high incidence area of thalassemia, with many reports of thalassemia in Guizhou, but there have been no reports of a large-scale investigation of abnormal hemoglobin phenotype and genotype. The aim of this study, therefore, was to investigate the phenotype and genotype of abnormal hemoglobin in a large cohort of people in Guizhou, China.

Materials and Methods

Subjects

The study cohort comprised 19,976 individuals from Guizhou Province, enrolled from October 2019 to January 2023, who underwent thalassemia screening at Guizhou Provincial People's Hospital. Three tubes of EDTA-anticoagulated venous blood were collected from each individual for routine blood evaluation, hemoglobin electrophoresis, and detection of common α and β thalassemia gene mutation types.

Hematology Test

Routine blood examination was performed using an automatic hematology analyzer (Sysmex, XN-9000, Kobe Japan), and the hemoglobin concentration (Hb), erythrocyte mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were recorded. Hemoglobin electrophoresis was performed using an automatic hemoglobin capillary electrophoresis instrument (Sebia, Capillarys2, Paris, France) to assess the concentrations of Hb A, Hb A_2, Hb F and any abnormal Hbs.

Thalassemia Gene Detection

Genomic DNA was extracted from 2 mL of EDTA anticoagulated blood with TIANamp Genomic DNA Kit (Tiangen Biotech,TIANGEN, Beijing, China) according to the manufacturer's recommendations. α and β thalassemia gene detection kits and matched automatic nucleic acid hybridization instruments (Hybriobio Limited, HBHM-3000S, Guangzhou, China) were used to detect six common α-thalassemia mutations ($-^{SEA}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, Hb CS CD142 TAA > CAA, Hb QS CD125 CTG > CCG, Hb WS CD122 CAC > CAG) and 17 β-thalassemia mutations (CD41-42 -TCTT, CD43 G > T, IVS-II-654 C > T, CD17 A > T, CD14-15 + G, −28 A > G, −29 A > G, CD71-72 + A, βE G > A, IVS-I-1 G > A/T, CD27-28 + C, IVS-I-5 G > C, CAP A > C or -AAAC, Int T > G, CD31-C, −30 T > C and −32 C > A).

DNA Sequencing

Sanger sequencing of *HBA1, HBA2* and *HBB* genes was performed in those with abnormal bands or increased normal band content on electrophoresis. From the Genbank database (www.ncbi.nlm.nih.gov), the reference sequences of HBA1, HBA2, and *HBB* were obtained, and primers were designed using Primer 5.0 software and synthesized by BiOligo Biotechnology (BiOligo Biotech, Shanghai, China) to amplify the *HBA1, HBA2*, and *HBB* genes, respectively. Primer sequences are shown in Table 1. The amplified products were sequenced with a sequencer (ABI, 3730XL, MA, USA), and then the sequencing results were compared with the reference sequences to identify the mutations leading to abnormal hemoglobin.

Pathogenicity Analysis of Emerging Abnormal Hemoglobin

Using Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) for conservative mutation amino acid analysis, and using Phyre2 (http://www.sbg.bio.ic.ac.uk) and SWISS-PDB Viewer4.10 for protein three dimensional structure prediction and analysis, the sample was analyzed for pathogenicity according to ACMG (American College of Medical Genetics and Genomics) guidelines.

Results

Of 19,976 individuals screened for thalassemia, 84 cases (detection rate, 0.42%) of abnormal hemoglobin were detected, all of which were heterozygous. There were 15 cases (18%) of α-globin variants which included 10 types: Hb Port Phillip, Hb I, Hb Orbassano, Hb Q-Thailand, Hb J-Toronto, Hb J-Norfolk, Hb G-Waimanalo, Hb Beijing, Hb Hekinan II and Hb Guiyang; Hb Port Phillip was the most common. The positions of *HBA* gene mutations, amino acid changes, abnormal hemoglobin electrophoresis bands, and contents of α-globin variants are shown in Table 2.Hematological characteristics of hemoglobin

Table 1 Primers Used for Sanger Sequencing of HBA1, HBA2 and HBB Gene

Gene	Primers	Primer sequence (5'→3')	Product (bp)
HBA1	a1F	CTCCGCGCCAGCCAATGAG	1020
HBA1	a1R	AGCTGCAGAGAGGTTCTAGCCAT	
HBA2	a2F	CTCCGCGCCAGCCAATGAG	1006
HBA2	a2R	CAGCTGCAGAGAGGTCCTTGGTC	
HBB	B1F	CGGCTGTCATCACTTAGACCT	582
HBB	B1R	CAGCTCACTCAGTGTGGCAAA	
HBB	B2F	GCTGTTATGGGCAACCCT	818
HBB	B2R	TTGCTATTGCCTTAACCCAGA	
HBB	B3F	ATGTATCATGCCTCTTTGCAC	587
HBB	B3R	GTTTTAAATGCACTGACCTCC	

Table 2 α Variation Types, Electrophoresis Bands and Average Content of Globin Chain

α globin variant	α gene mutation	Amino acid changes	n	%	Zone and content %
Hb Port Phillip	*HBA2*:c.275T>C	91 (FG3) Leu>Pro	4	26.67	Z8,7.8
Hb I	*HBA2*:c.49A>G	16 (A14) Lys>Glu	2	13.33	Z15,21
Hb Orbassano	*HBA2*:c.46G>T	15 (A13) Gly>Cys	2	13.33	Z13,15.1
Hb Q-Thailand	*HBA1*:c.223G>C	74 (EF3) Asp>His	1	6.67	Z(F),28.2
Hb J-Toronto	*HBA2*:c.17C>A	5 (A3) Ala>Asp	1	6.67	Z12,24.2
Hb J-Norfolk	*HBA2*:c.173G>A	57 (E6) Gly>Asp	1	6.67	Z13,32.1
Hb G-Waimanalo	*HBA2*:c.193G>A	64 (E13) Asp>Asn	1	6.67	Z(D),25
Hb Beijing	*HBA2*:c.51G>T	16 (A14) Lys>Asn	1	6.67	Z13,24.5
Hb Hekinan II	*HBA1*:c.84G>T	27 (B8) Glu>Asp	1	6.67	Z(A),97.7
Hb Guiyang	*HBA2*:c.151C>A	50 (CE8) His>Asn	1	6.67	Z12,23.6

with abnormal α globin chain are shown in Table 3. Sixty-nine cases (82%) of β-globin variation were detected, which included 10 types: Hb E, Hb New York, Hb J Bangkok, Hb D-Punjab, Hb Lome, Hb G-Taipei, Hb G-San José, Hb Hope and Hb Mizuho; Hb E (50/84) was the most common, followed by Hb New York (10/84). The positions of *HBB* gene mutations, amino acid changes, abnormal hemoglobin electrophoresis bands and contents of β-globin variants are shown in Table 4. Hematological characteristics of hemoglobin with abnormal β globin chain are shown in Table 5.

In the present study, a novel abnormal hemoglobin (HBA2: c.151C > A) was detected, named Hb Guiyang because it was first identified in Guiyang city. The individual with Hb Guiyang was a 29-year-old woman who had red cell indices of RBC 4.45×10^{12}/L, Hb 136 g/L, MCV 90.6 fL, MCH 30.6 pg; hemoglobin electrophoresis results of HbA 74.3%, HbA_2 2.1%, and abnormal hemoglobin 23.6% in Z12 region (Figure 1). Her thalassemia screening genotypes for α- and β-thalassemia were αα/αα and β^N/β^N, respectively, and Sanger sequencing results showed that she was a heterozygous carrier of HBA2: c.151C > A (Figure 2). The conserved analysis of mutant amino acids in six species was highly conserved, and the three-dimensional structure prediction of the protein showed no change in hydrogen bonds and only charge changes (Figure 3). The pathogenicity of *HBA2*: c.151C > A was a variant of uncertain significance (VUS) as evidenced by PM2 according to the ACMG guidelines. In addition, one case of Hb Mizuho (*HBB*: c.206T >C) was detected in a 2-year-old girl with severe hemolytic anemia, the first time Hb Mizuho was found in a Chinese population.

Among the 15 cases of abnormal α-chain hemoglobin, 12 were heterozygotes only, none of whom had clinical manifestations, their Hb content was above 110 g/L, and both their MCV and MCH were in the normal range. Two heterozygote cases of abnormal α-chain hemoglobins with silent α-thalassemia had no anemia phenotype, but their MCV and MCH were below the normal range.The study identified a novel mutation for α-thalassaemia and Hb Portland that is

Table 3 Hematological Characteristics of Hemoglobin with Abnormal α Globin Chain

α globin variant	n	Hb(g/L)	MCV (fl)	MCH(pg)
Hb Port Phillip heterozygote	2	111	82.3	27.5
Hb Port Phillip heterozygote Combined iron deficiency	1	62	72	27.6
Hb Port Phillip heterozygote/-$\alpha^{3.7}$	1	121	79.3	24.5
Hb I heterozygote	2	126	92	29.9
Hb Orbassano heterozygote	2	145.5	92.4	32.7
Hb Q-Thailand heterozygote/-$\alpha^{4.2}$	1	143	78.9	26.3
Hb J-Toronto heterozygote	1	158	88.6	31.6
Hb J-Norfolk heterozygote	1	155	88.3	29.3
Hb G-Waimanalo heterozygote	1	178	84.9	29.7
Hb Beijing heterozygote	1	124	94.6	31.7
Hb Hekinan II heterozygote	1	110	88.4	26.9
Hb Guiyang heterozygote	1	136	90.6	30.6

Table 4 β Variation Types, Electrophoresis Bands and Average Content of Globin Chain

β globin variant	β gene mutation	Amino acid changes	n	%	Zone and content %
Hb E	*HBB*:c.79G>A	26 (B8) Glu>Lys	50	72.5	Z(E),25.2
Hb New York	*HBB*:c.341T>A	113 (G15) Val>Glu	10	14.5	Z11,42.8
Hb J Bangkok	*HBB*:c.170G>A	56 (D7) Gly>Asp	2	2.9	Z12,46.7
Hb D-Punjab	*HBB*:c.364G>C	121 (GH4) Glu>Gln	1	1.5	Z(D),40.7
Hb J-Lome	*HBB*:c.180G>C	59 (E3) Lys>Asn	1	1.5	Z13,50.7
Hb G-Taipei	*HBB*:c.68A>G	22 (B4) Glu>Gly	1	1.5	Z(D),38.4
Hb G-Coushatta	*HBB*:c.68A>C	22 (B4) Glu>Ala	1	1.5	Z(D),40
Hb G-San José	*HBB*:c.23A>G	7 (A4) Glu>Gly	1	1.5	Z(F),31.9
Hb Hope	*HBB*:c.410G>A	136 (H14) Gly>Asp	1	1.5	Z10,35.2
Hb Mizuho	*HBB*:c.206T>C	68 (E12) Leu>Pro	1	1.5	Z(F),18.9

Table 5 Hematological Characteristics of Hemoglobin with Abnormal β Globin Chain

β globin variant	n	Hb (g/L)	MCV (fl)	MCH(pg)
Hb E heterozygote	41	121.5±16	78±5.8	26±2.2
Hb E heterozygote combined with iron deficiency	4	84.8±12.2	72.2±8.2	21.7±2.6
Hb E heterozygote combined with $-\alpha^{3.7}/\alpha\alpha$	2	138.5	82.3	28
Hb E heterozygote combined with $--^{SEA}/\alpha\alpha$	1	121	66.2	20.6
Hb E heterozygote/β^{CD17}	1	73	86.3	25.6
Hb E heterozygote/$\beta^{CD27-28}$	1	59	77.6	24.5
Hb New York heterozygote	8	129.5±3.4	86.5±3.2	29.1±1.9
Hb New York heterozygote combined with iron deficiency	1	94	76.9	23.1
Hb New York heterozygote combined with $--^{SEA}/\alpha\alpha$	1	112	63.7	20.6
Hb J Bangkok heterozygote	1	124	97.6	32.9
Hb J Bangkok heterozygote combined iron deficiency	1	64	78.8	21.5
Hb D-Punjab heterozygote	1	114	108.2	36.7
Hb J-Lome heterozygote	1	132	89.5	31.4
Hb G-Taipei heterozygote	1	132	84.4	28.2
Hb G-Coushatta heterozygote	1	124	84.6	29.3
Hb Mizuho heterozygote	1	67	99.1	28.6
Hb G-San José heterozygote combined iron deficiency	1	97	79.6	23.5
Hb Hope heterozygote combined with $--^{SEA}/\alpha\alpha$	1	107	69.3	22.5

not common in Southwest China. One heterozygote case of abnormal α-chain hemoglobin with iron deficiency anemia had significant anemia manifestations (Hb: 62 g/L), and MCV and MCH were also below the normal range.

Among the 69 cases of abnormal β-chain hemoglobin, 55were heterozygotes only, including 41 Hb E heterozygotes, and their mean hemoglobin content was normal (121.5 ± 16 g/L), but their MCV and MCH were below the normal range. One heterozygote case of Hb Mizuho had significant manifestations of anemia (Hb: 67 g/L), with both MCV and MCH in the normal range. The other 13 heterozygotes of abnormal β-chain hemoglobin had no clinical phenotype, and their hemoglobin content, MCV and MCH were within the normal range. Seven heterozygote cases of abnormal β-chain hemoglobin with iron deficiency anemia showed moderate to severe anemia, and their MCV and MCH were also below the normal range. There were three heterozygote cases of abnormal β-chain hemoglobin with α-thalassemia minor, of which two cases had no anemia, one case had mild anemia, and the MCV and MCH of three cases were below the normal range. Two Hb E heterozygotes with silent α-thalassemia had no clinical phenotype, and both their MCV and MCH were within the cut-off range. Two Hb E heterozygotes with β-thalassemia minor showed moderate to severe anemia, and their MCV and MCH were below the normal range.

Discussion

In this study, 84 cases of abnormal hemoglobin were detected in 19,976 Guizhou patients with thalassemia screening, including 15 cases of α globin chain and 69 cases of β globin chain, with a total detection rate of 0.42%, higher than the carrier rate of 0.2% in Guizhou in the 1980s.[8] It was lower than 0.78% in Yunnan,[9] 0.59% in Guangxi,[10] 0.57% in Chongqing,[11] 0.49% in Hunan,[12] and 0.44% (excluding Hb E) in Guangxi, Yunnan-Guizhou junction,[13] which may be related to regional and population differences.

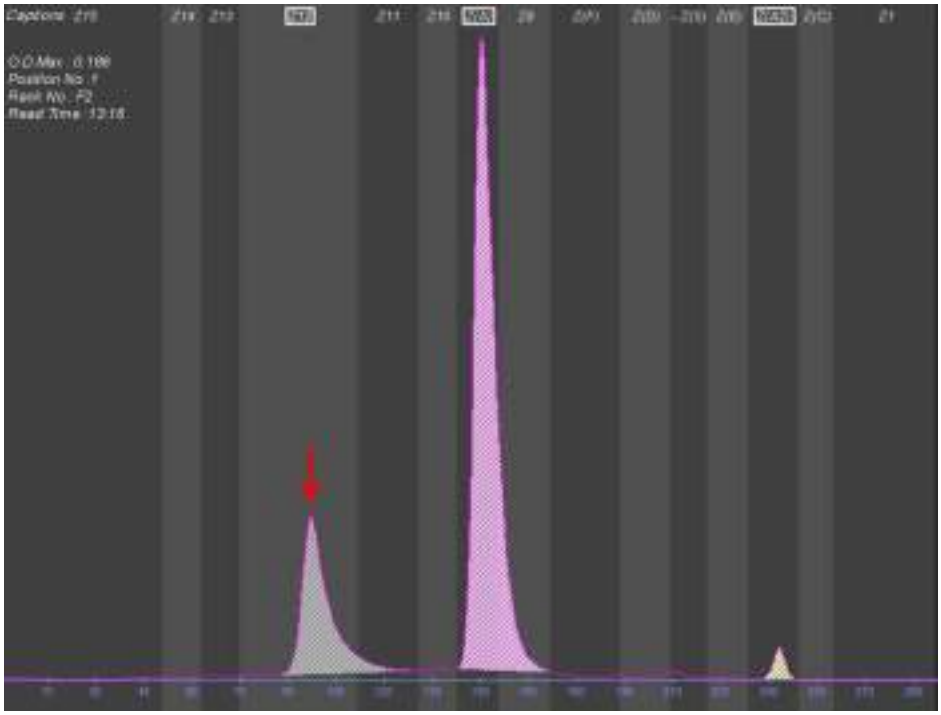

Figure 1 Hemoglobin capillary electrophoresis of the patient with Hb Guiyang.
Notes: The red arrow indicates the area where abnormal hemoglobin Hb Guiyang is located.

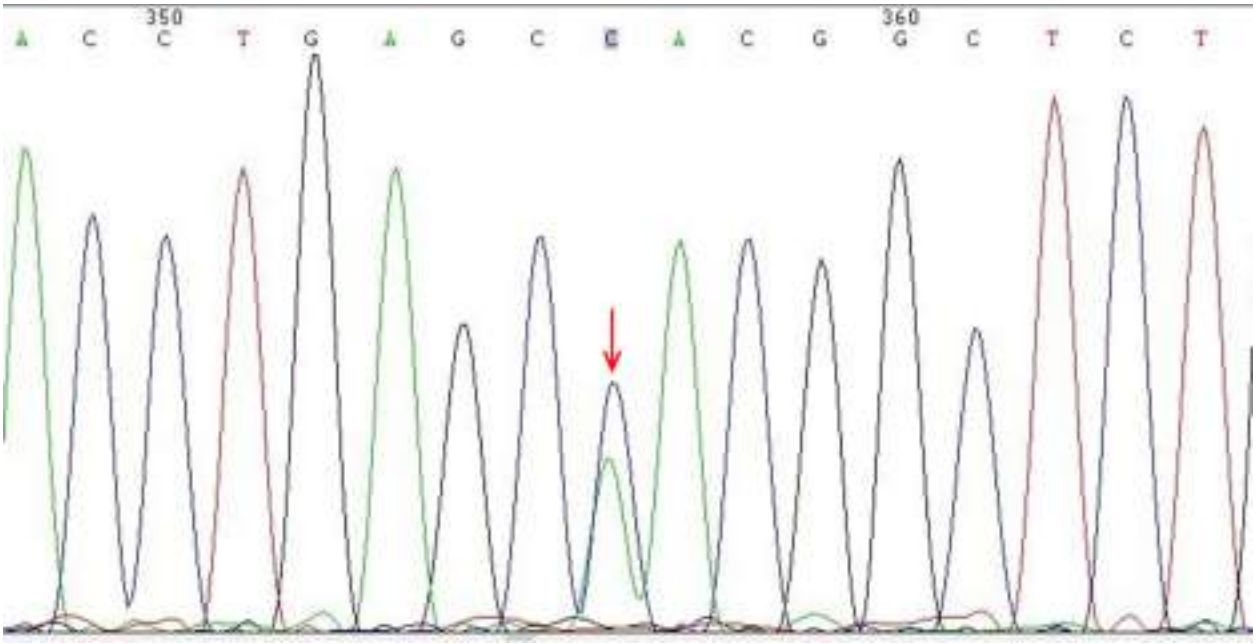

Figure 2 Sanger sequencing peak of *HBA2* gene in patients with Hb Guiyang.
Notes: The red arrow in the sequencing graphs indicate the position of the HBA2: c.151C > A mutation.

According to reports in the 1980s, there were differences in the type of abnormal hemoglobin between the South and North of China; the most common abnormal hemoglobins in the South were Hb E, Hb New York, Hb G Chinese, Hb Q Thailand, and Hb J Bangkok; the most common abnormal hemoglobin in the North was Hb D Punjab.[8] In recent years, a large screening sample (311,042 people) in Southern China found the detection rate of abnormal hemoglobin was

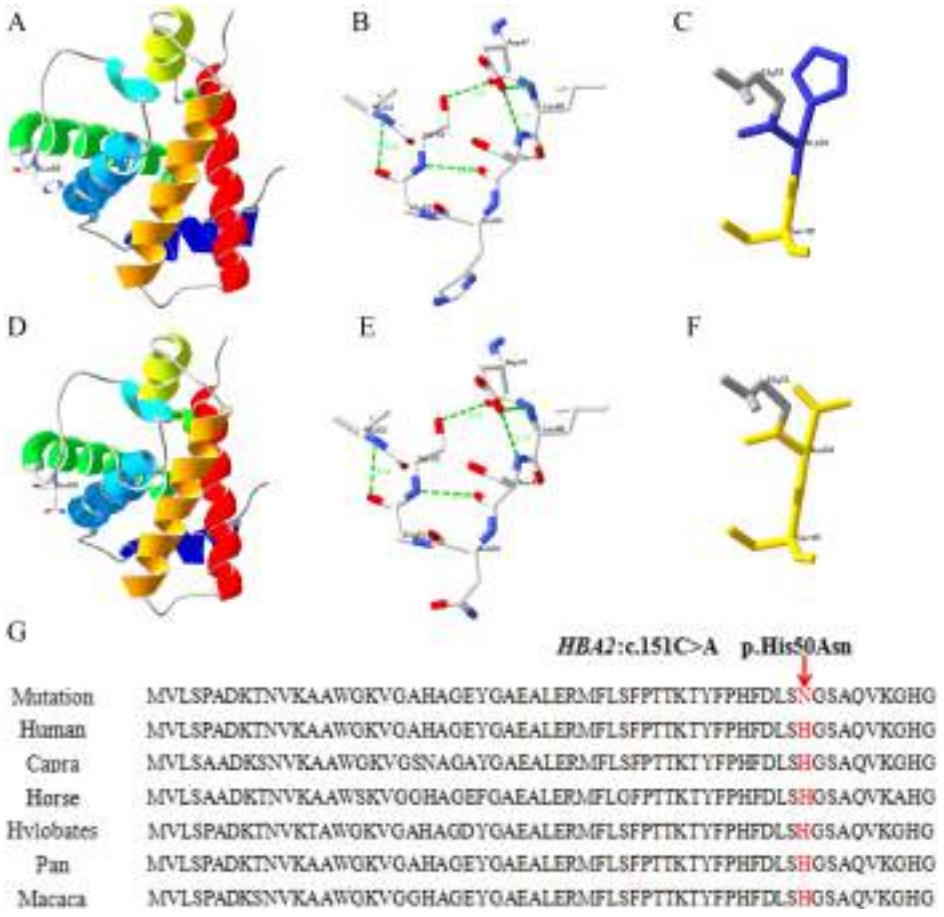

Figure 3 Protein three-dimensional structure prediction and conservation analysis of the Hb Guiyang. (**A, B** and **C**) are the three dimensional structure prediction and the partial enlargement of wild-type protein. (**D, E** and **F**) are the mutant protein. (**G**): the positions p.His50 in the HBA2 protein are highly conserved among six species.

0.35% (117 people), and the most common abnormal hemoglobins were: Hb E, Hb New York, Hb J Bangkok, and Hb Q Thailand.[14] In the present study, 20 types of abnormal hemoglobin were detected, including 10 kinds each of α and β globin chains, and the three most common abnormal hemoglobins were Hb E (50/84), Hb New York (10/84), and Hb Port Phillip (4/84). Hb E is undoubtedly the most common abnormal hemoglobin in Southern China, followed by Hb New York, consistent with previous reports. Surprisingly, however, four cases of Hb Port Phillip were found in this study, and only a few cases have been reported in the literature worldwide,[14–18] indicating that Hb Port Phillip is surprisingly not rare in Guizhou. Additionally, abnormal hemoglobin Hb Mizuho, Hb Orbassano, Hb J-Toronto, and Hb J-Norfolk were also reported for the first time in the Chinese population, and abnormal hemoglobin Hb Beijing and Hb G-San José were reported for the first time in the Guizhou population of China.

By querying the HbVar database, this study found an abnormal hemoglobin (HBA2: c.151C > A) that had not been previously reported worldwide; it was caused by the substitution of histidine by asparagine at codon 50 CAC > AAC of the α2 globin gene. According to international nomenclature, this abnormal hemoglobin was named Hb Guiyang. In this case, the newly identified abnormal hemoglobin carriers had a normal hematological phenotype, and 23.6% of the abnormal peak profiles appeared in the Z12 region only in hemoglobin capillary electrophoresis. Although the conservation analysis of amino acids at the mutation point was highly conserved, protein structure prediction showed that only the charge of amino acids at the mutation point was changed, and amino acids before and after mutation were polar amino acids, consult NCBI (https://www.ncbi.nlm.nih.gov). This mutation site is known not to be located in the position where it binds to the heme moiety, and not at the tetramer contact surface; it is speculated that it does not affect protein structural stability. Combined with the pathogenicity analysis of ACMG, together with the abnormal hemoglobins Hb

South Yorkshire (HBA2: c.151C > T) and Hb J-Sardegna (HBA2: c.151C > G) being caused by different base substitutions at the same locus but with no clinical manifestations, in the present case, it was comprehensively speculated that this mutation would not cause abnormal hemoglobin function.

Most abnormal hemoglobins have only mild clinical manifestations or none at all because their mutated amino acids are located outside the hemoglobin molecule. Nevertheless, a few can cause moderate to severe anemia, especially referring to certain abnormal hemoglobin homozygotes, combined thalassemia, or other abnormal hemoglobins. Concordant with this study, Hb E heterozygotes alone have been shown to cause mild microcytic hypochromia with or without anemia, and there were two cases of significant anemia when Hb E was combined with β^0, which was due to the fact that Hb E/β^0 had little β-chain synthesis, there was little HbA production, and hemoglobin naturally decreased.[19,20] One case of Hb Hope complex–^{SEA}/αα showed mild microcytic hypochromic anemia, consistent with Pornprasert's report.[21] Although Hb Port Phillip has been documented on HbVar to cause hemoglobin instability due to loss of the heme interface, Hb Port Phillip heterozygotes alone in this study did not have significant manifestations of anemia and were only significantly anemic when combined with –^{SEA}/αα, consistent with the findings of Du et al.[17] Because Hb Port Phillip is rarely reported in the literature, the study of phenotype needs to be further accumulated. From the literature and our previous report,[22] Hb Mizuho caused severe hemolytic anemia because the mutated amino acid affected the binding of distal histidine β63 (E7) to the heme moiety. Relative to clinical management of unexplained anemia, DNA sequencing of the globin gene should be performed to identify the cause even if routine hemoglobin component tests reveal no abnormalities.

Conclusion

In summary, this study confirmed that there were a wide variety of abnormal hemoglobin species in Guizhou, China. This study enriched the molecular epidemiological data of abnormal hemoglobinopathy in Guizhou, and also provided reference data for genetic counseling and prenatal diagnosis in Guizhou.

Ethics and Consent Statements

This study was approved by the Medical Ethics Committee at Guizhou Provincial People's Hospital (approval number 2022-05). We obtained informed consent forms from all subjects or guardians. We confirm that our study complies with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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ORIGINAL RESEARCH

Haematological Indices in Acute Coronary Syndrome Patients in Ethiopia: A Comparative Cross-Sectional Study

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Background: Numerous biomarkers are used as diagnostic, prognostic, and predictive indicators of myocardial ischemia. The most commonly used biomarkers are cardiac troponin I (Tn-I) and creatinine kinase (CK-MB). However, in developing nations, their availability in primary care settings is extremely limited. In such situations, easily available assays such as complete blood count (CBC) should be investigated as prognostic indicators in individuals with acute coronary syndrome (ACS).

Objective: This study aimed to compare the pattern of haematological indices and blood cell ratios of ACS patients compared with apparently healthy controls.

Methods: Patients diagnosed with ACS were recruited consecutively between 01 May 2022 and 31 October 2023 at Jimma Medical Center (JMC). Biochemical analyses and complete blood counts were performed. Analysis of variance was performed to compare the continuous variables. Spearman correlation coefficient tests were performed to correlate hematologic parameters with high sensitive troponin-I (hs-Tn-I) levels.

Results: This study enrolled 220 participants (110 patients with ACS and age, sex, and place of residence matched 110 non-ACS controls). From ACS group 99 (90%) were diagnosed with ST-elevated myocardial infarction. The ACS group had a significantly greater mean platelet volume (MPV), white blood cell count, red cell distribution width (RDW), neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio. The RDW ($r = 0.248$, $p = 0.009$) and MPV ($r = 0.245$, $p = 0.009$) were significantly positively correlated with hs-Tn-I levels in the ACS group. MPV, RDW, and monocyte count were significantly higher in non-survivor ACS patients ($p < 0.05$).

Conclusion: The significant differences observed in haematological parameters between individuals with ACS and healthy controls suggest the potential utility of these easily accessible and cost-effective diagnostics in predicting future morbidity and ACS risk. Incorporating these routine evaluations into clinical practice could enhance risk assessment and improve patient outcomes.

Keywords: acute coronary syndrome, haematological indices, ACS prognosis, mortality risk, Jimma medical center

Introduction

Acute coronary syndrome (ACS) is a collection of signs and symptoms induced by plaque rupture and platelet-rich coronary thrombosis. Thrombus produces partial or full coronary artery obstruction, resulting in myocardial ischemia and numerous clinical symptoms, ranging from unstable angina (UA) to myocardial infarction, which are common causes of mortality in developing nations.[1] Biomarkers can reflect the pathophysiological process of ACS, provide expressive information about prognosis, and support clinical decision-making without replicating any clinically available information. Numerous ACS biomarkers are currently available and clinically used as diagnostic, prognostic, or predictive indicators.[2] The most commonly used biomarkers are high-sensitivity troponin T or I (hs-cTn) and creatinine phosphokinase MB (CK-MB).[3] Numerous studies have shown a strong correlation between haematological parameters and the

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risk of unfavorable outcomes in patients with ACS because of systemic hypoxemia and inflammation linked to pathophysiological mechanisms. Depending on the severity of the injury, this can lead to circulatory failure and, ultimately, organic dysfunction.[4]

The inflammatory processes and increased myeloid cell activity linked to ACS resulted in a rise in erythropoietin levels. Haematological parameters in peripheral blood can be used to diagnose the condition. The onset and progression of atherosclerotic plaques are significantly influenced by low levels of chronic inflammation, which results in the instability of the plaque and the production of thrombus.[5]

Neutrophils are the first class of leukocytes to be found in damaged areas. Upon activation, a substantial quantity of inflammatory mediators are generated, which regulate the reaction to tissue damage by exhibiting proteolytic enzymes, hypoxic damage, and other mediators.[6] Cytokines (interleukin III, interleukin VI, and thrombopoietin) are essential for controlling megakaryocyte ploidy and platelet quantity during the formation of larger-volume platelets.[7] Leukocyte recruitment to atherosclerotic lesions is boosted by platelet–leukocyte interactions, which also excite neutrophils and lead to the formation of neutrophil extracellular traps, which bind platelets and accelerate atherogenesis and atherothrombosis.[8] A quick and easy way to determine a patient's inflammatory condition is to use the neutrophil-to-lymphocyte ratio (NLR). Its value has been demonstrated in the stratification of mortality in the majority of cardiac events, as well as in the prediction and marking of infectious or inflammatory diseases and problems following surgery.[9] The heterogeneity of red blood cell size is measured by red cell distribution width (RDW), which is derived from the RBC size distribution curves. It has been demonstrated that this measure can predict morbidity and death in a number of cardiovascular conditions, including acute myocardial infarction, stable coronary artery disease, and heart failure.[10,11] Additionally, RDW has been suggested as a stand-alone predictor of mortality for patients with non-ST-elevation myocardial infarction (NSTEMI).[12]

Despite the overwhelming number of studies on hematologic biomarkers and cardiovascular disease, there is a paucity of published comprehensive studies on the relevance and implications of haematological parameters for ACS in low-income settings in sub-Saharan Africa, particularly where anemia and infection due to parasites, bacteria, mycobacteria, and viruses are common and affect hematologic biomarkers. Thus, this study assessed the haematological indices between patients with ACS and an apparently healthy control group.

Materials and Methods

Research Design and Data Collection

This study was conducted in the cardiovascular unit of the Jimma Medical Center (JMC) for 18 consecutive months (May 1, 2022, to October 31, 2023). JMC is one of the largest and oldest teaching and referral hospital in southwest Ethiopia. An institution-based comparative cross-sectional study was implemented to determine the pattern of haematological indices among patients with ACS and an apparently healthy comparative group. Consecutive patients admitted with confirmed ACS (based on electrocardiography, cardiac biomarkers, and clinical symptoms) during the study period were included. Patients aged less than 18 years and those who had been diagnosed with a haematological disease, known malignancy, systemic inflammatory or autoimmune disease, thrombocytopenia before admission, secondary anemia, chronic liver disease, renal failure, immunosuppressive therapy, use of anticoagulant agents, and readmission after discharge were excluded. Age-, sex-, and place of residence-matched apparently healthy individuals (a person without sign and symptoms of ACS and free from any chronic diseases listed in the exclusion criteria) from the Jimma community, Jimma University workers, patient visitors, and patient attendants were recruited purposively in a 1–1 ratio as the non-ACS comparative group.

After obtaining written informed consent, the participant's medical records were assessed for the possibility of exclusion criteria. Then, face-to-face interviews were conducted with both groups using a structured questionnaire. Data on the demographic, health-related, clinical, and outcome status of patients with ACS were recorded. Venous blood samples were collected under aseptic conditions using a disposable syringe; 2 mL of it was placed in an ethylenediaminetetraacetic acid (EDTA) tube for complete blood count analysis and the remaining 2 mL in a vacuum tube (organ tube) for serum biochemical analysis. Trained laboratory professionals performed the complete blood count (CBC) and serum biochemical analyses. Complete blood counts, including hemoglobin, red blood cells (RBC), RDW, white blood cells (WBC), WBC differential

counts (neutrophils, lymphocytes, eosinophils, basophils, and monocytes), platelet count (PLC), mean platelet volume (MPV), Plateletcrit (PCT), and percentages, were analyzed using the Uni-CelDxH 800 Coulter Cellular Analysis System. The NLR, platelet-to-lymphocyte ratio (PLR), MPV-to-lymphocyte ratio (MPVLR), and WBC-to-MPV ratio (WMR) were calculated. A Roche Cobas Integra 400 analyzer was used to detect essential serum biochemical parameters, including creatinine levels and high-sensitivity troponin I. All measurements were performed within 30 minutes of blood collection. Blood sample quality and analyses were performed using the standard operating procedures.

Statistical Analysis

Stata-SE version 14 was used to analyze the data. The report format for continuous variables was mean ± SD. Categorical variables were described using percentages and definite values. A *t*-test or ANOVA was computed to compare continuous variables, and the Mann–Whitney U and Kruskal–Wallis tests were applied to assess values that were not normally distributed. Variables between categories were compared using the chi-square and Fisher's exact test. When the p-value was less than 0.05, differences were regarded as statistically significant. The correlation between the two continuous parameters was calculated using Spearman correlation coefficient.

Results

This study enrolled 220 participants (110 patients with ACS and 110 controls without ACS (non-ACS)). Of the 110 ACS patients, 74 (67.3%) were men, indicating a significantly higher population of men among ACS-admitted patients. In the study group, the final diagnoses for 99 (90%), 9 (8.2%), and 2 (1.8%) patients were STEMI, NSTEMI, and unstable angina (UA), respectively (Figure 1). The mean age of the ACS patients was 56.69 ± 11.9 years. The majority of ACS patients, 90 (81.8%), were discharged from the hospital with improvement, while 20 (18.2%) died (Figure 2). Some demographic and health-related factors were significantly different between the ACS patients and non-ACS controls. Age, sex, and place of residence were matched between the two groups. Higher levels of education and regular exercise participation were two characteristics that revealed notable disparities between the two groups (Table 1).

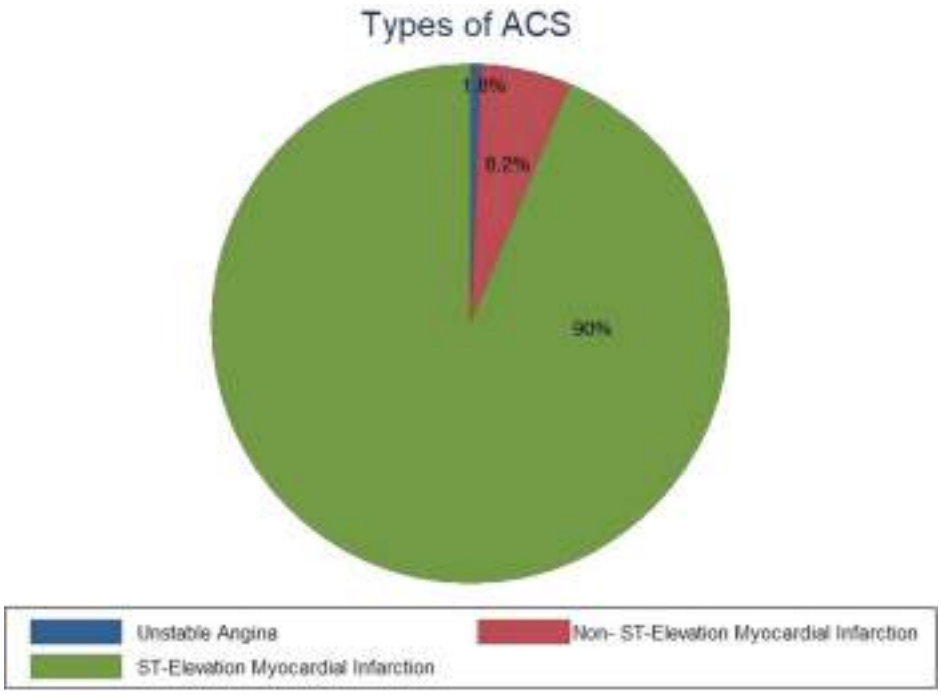

Figure 1 Types of acute coronary syndrome.

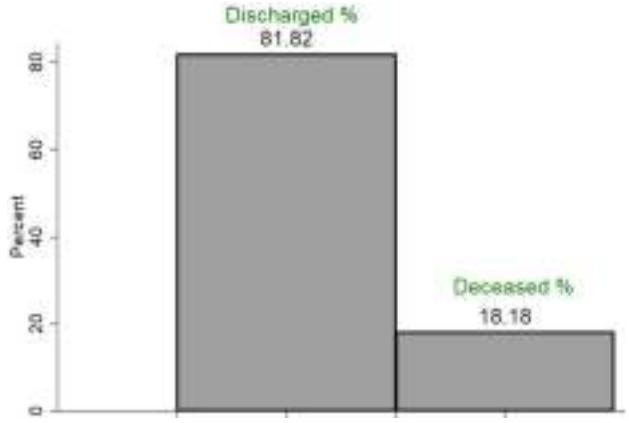

Figure 2 Acute coronary syndrome patients' outcome.

Analysis of differences in hematological indices and troponin levels between the ACS patients and non-ACS control groups showed significantly higher values in the parameters of WBC, MCHC, RDW, PLC, MPV, neutrophils, basophils, NLR, PLR, WBC-to-MPV ratio, MPVLR, hs-troponin I, and plateletcrit in the ACS group, whereas RBC, HGB, HCT, lymphocytes, and eosinophils were significantly higher in the non-ACS control groups (p <0.05). Table 2 displays the variations in each hematological parameter values between the two groups.

Correlations between haematological indices and highly sensitive troponin-I, the gold standard for ACS diagnosis, were computed in both study groups separately. There were no significant positive or negative correlations in the non-ACS control group, whereas RDW%, RDW-SD, and MPV were significantly positively correlated with hs-Tn-I (Table 3 and Figures 3 and 4).

Table 1 Demographics and Health-Related Characteristics of the Study Participants

Variables		ACS Case N (%)		Non-ACS Control N (%)		*p*-value
Age in Years	Mean (SD)	56.69 ± 11.91		56.5 ± 11.80		0.905
Sex	Male	74	67.27	74	67.27	1.000
	Female	36	32.73	36	32.73	
Residence	Urban	60	54.55	60	54.55	1.000
	Rural	50	45.45	50	45.45	
Educational status	Do not read and write	37	33.64	21	19.09	0.022
	Primary	25	22.73	38	34.55	
	Secondary	18	16.36	27	24.55	
	Tertiary	30	27.27	24	21.82	
Current cigarettes smoker	Yes	2	1.82	8	7.27	0.052
	No	108	98.18	102	92.73	
Regular exercise	Yes	25	22.73	94	85.45	0.000
	No	85	77.27	16	14.55	
Diabetes Mellitus	Yes	33	30.00	20	18.18	0.6855
	No	77	70.00	90	81.18	
Hypertension	Yes	50	45.45	25	22.73	0.2616
	No	60	54.54	85	77.27	
Family History of ACS	Yes	13	11.82	10	9.09	0.2554
	No	97	88.18	100	90.91	
BMI (kg/m^2)	*Mean (SD)*	24.0 ± 3.5		27.7 ± 29.1		0.194
WHR	*Mean (SD)*	1.0 ± 0.09		1.0 ± 0.1		0.100

Abbreviations: BMI, Body mass index; WHR, Waist-to-hip ratio.

Table 2 Comparison of Haematological Indices and Biochemical Markers Between ACS and Non-ACS Comparative Groups

Hematologic Indices	ACS Case Mean ±SD	Non-ACS Control Mean ±SD	*p*-value
White blood cell count 10^3 /μL, (Mean ±SD)	10.5±4.8	8.0±3.4	0.001
Red blood cell count 10^6/μL,(Mean ±SD)	4.7±0.9	5.0±0.6	0.007
Hemoglobin gm/dl, (Mean ±SD)	13.6±2.7	14.4±1.7	0.009
Hematocrit- % (Mean ±SD)	40.8±7.7	44.4±5.7	0.001
MCV fl, (Mean ±SD)	87.7±7.8	88.8±6.7	0.278
MCH pg, (Mean ±SD)	29.1±3.0	28.8±1.8	0.345
MCHC mg/dl, (Mean ±SD)	32.97±2.4	32.34±1.9	0.031
Red cell distribution width-CV (%)	19.5±2.2	14.4±1.8	0.001
RDW-SD fl, (Mean ±SD)	62.1±7.2	45.4±8.0	0.001
Platelet count 10^3 /μL, (Mean ±SD)	254.6±134.5	236.7±75.1	0.224
MPV fl, (Mean ±SD)	11.8±1.1	10.6±1.4	0.001
Neutrophil count 10^3 /μL (Mean ±SD)	8.1±4.4	5.2±3.2	0.001
Lymphocyte count 10^3 /μL (Mean ±SD)	1.2±0.7	2.0±0.8	0.001
Monocyte count 10^3 /μL (Mean ±SD)	0.6±0.5	0.5±0.3	0.060
Eosinophil count 10^3 /μL (Mean ±SD)	0.1±0.2	0.2±0.2	0.001
Basophil count 10^3 /μL (Mean ±SD)	0.07±0.1	0.01±0.06	0.001
Neutrophil to lymphocyte ratio (Mean ±SD)	12.0±22.9	3.4±5.2	0.001
Platelet to lymphocyte ratio (Mean ±SD)	313.0±457.2	135.4±92.0	0.001
WBC to MPV ratio (Mean ±SD)	0.9±0.4	0.8±0.4	0.030
MPV to lymphocyte ratio, (Mean ±SD)	15.2±18.3	6.2±4.2	0.001
Plateletcrit (%)	3.0±1.5	2.5±0.8	0.005
High Sensitive Troponin I (ng/L)	87.37±85.5	8.82±6.9	0.001

Abbreviations: MCV, Mean corpuscular volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width; MPV, Mean Platelet Volume.

Table 3 Correlations of Haematological Indices and Their Derivatives with High Sensitive Troponin-I

Hematologic Indices	ACS Case r	*p*-value	Non-ACS Control r	*p*-value
iWhite blood cell count 10^3 /μL, (Mean ±SD)	–0.024	0.804	–0.102	0.288
Red blood cell count 10^6/μL,(Mean ±SD)	0.136	0.157	–0.009	0.921
Hemoglobin gm/dl, (Mean ±SD)	0.064	0.508	0.033	0.725
Hematocrit- % (Mean ±SD)	0.068	0.483	–0.022	0.819
MCV fl, (Mean ±SD)	–0.125	0.191	0.011	0.903
MCH pg, (Mean ±SD)	–0.084	0.380	0.053	0.579
MCHC mg/dl, (Mean ±SD)	0.069	0.468	0.069	0.474
Red cell distribution width-CV (%)	0.248	0.009	–0.119	0.226
RDW-SD fl, (Mean ±SD)	0.189	0.047	–0.077	0.420
Platelet count 10^3 /μL, (Mean ±SD)	0.051	0.597	0.087	0.365
MPV fl, (Mean ±SD)	0.245	0.009	0.106	0.269
Neutrophil count 10^3 /μL (Mean ±SD)	0.003	0.969	–0.099	0.300
Lymphocyte count 10^3 /μL (Mean ±SD)	0.019	0.843	0.013	0.888
Monocyte count 10^3 /μL (Mean ±SD)	0.123	0.197	–0.073	0.446
Eosinophil count 10^3 /μL (Mean ±SD)	–0.068	0.478	–0.011	0.906
Basophil count 10^3 /μL (Mean ±SD)	–0.096	0.315	–0.098	0.310
Neutrophil to lymphocyte ratio (Mean ±SD)	–0.025	0.794	–0.134	0.162
Platelet to lymphocyte ratio (Mean ±SD)	–0.000	0.993	–0.070	0.465

(*Continued*)

Table 3 (Continued).

Hematologic Indices	ACS Case r	*p*-value	Non-ACS Control r	*p*-value
WBC to MPV ratio *(Mean ±SD)*	–0.078	0.413	–0.111	0.248
MPV to lymphocyte ratio, *(Mean ±SD)*	–0.018	0.844	–0.071	0.459
Plateletcrit (%)	0.113	0.237	0.110	0.254

Abbreviations: MCV, Mean corpuscular volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width; MPV, Mean Platelet Volume.

Haematological indices and biochemical markers based on patient outcomes were also compared. The mean differences in mean platelet volume (MPV), RDW%, RDW-SD, monocytes, creatinine, and hs-Tn-I were significantly higher in the non-survivor group, whereas the mean corpuscular volume (MCV) was significantly greater in the ACS survivor group (Table 4).

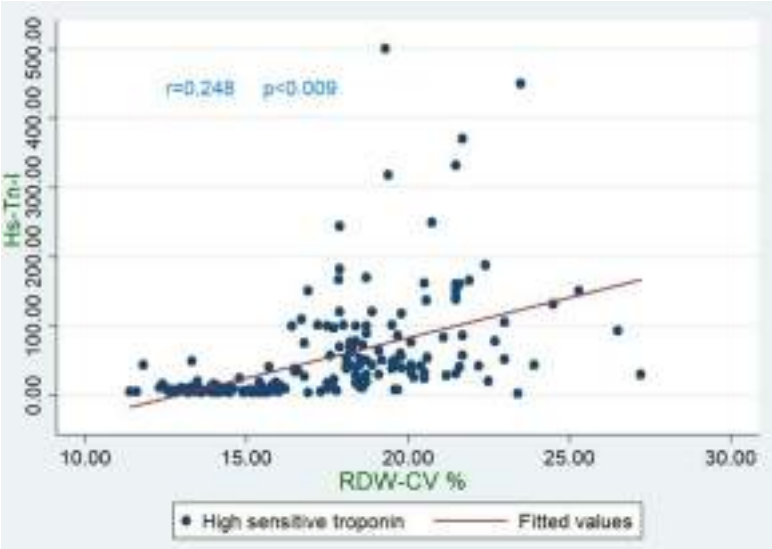

Figure 3 Correlation between RDW-CV % and high sensitive troponin-I.

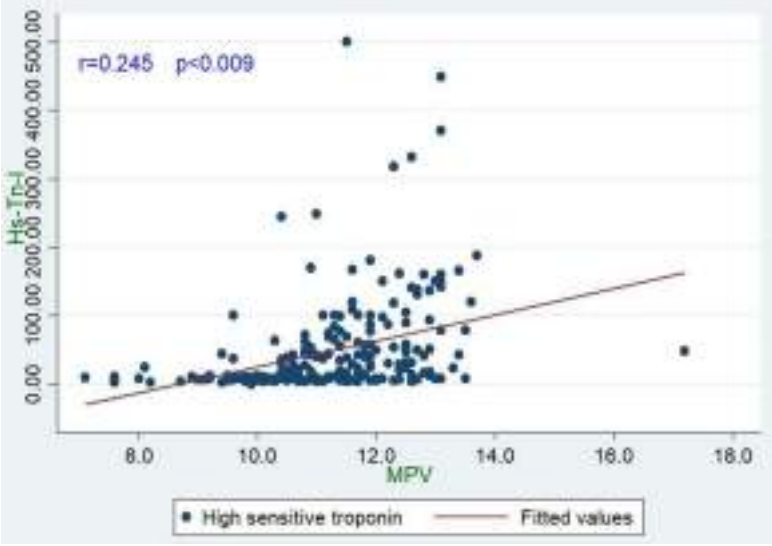

Figure 4 Correlation between MPV and high sensitive troponin-I.

Table 4 Comparison of Haematological Indices and Biochemical Markers Between ACS Survivors and Non-Survivor Groups

Hematologic Indices	ACS Survivors (N=90) Mean ± SD	ACS Non-Survivors (N=20) Mean ± SD	*p*-value
White blood cell count 10^3 /μL, (Mean ±SD)	10.198±4.8	12.035±4.5	0.122
Red blood cell count 10^6/μL,(Mean ±SD)	4.653±0.9	4.938±0.8	0.206
Hemoglobin gm/dl, (Mean ±SD)	13.551±2.6	13.716±2.7	0.805
Hematocrit- % (Mean ±SD)	40.780±7.8	41.159±7.4	0.843
MCV fl, (Mean ±SD)	88.671±7.9	83.515±6.3	0.007
MCH pg, (Mean ±SD)	29.356±3.1	27.968±2.0	0.058
MCHC mg/dl, (Mean ±SD)	32.906±2.4	33.280±1.9	0.526
Red cell distribution width-CV (%)	19.123±2.1	21.320±1.8	0.001
RDW-SD fl, (Mean ±SD)	60.976±7.0	67.164±5.8	0.001
Platelet count 10^3 /μL, (Mean ±SD)	256.598±143.2	245.750±87.3	0.745
MPV fl, (Mean ±SD)	11.658±1.1	12.425±0.9	0.005
Neutrophil count 10^3 /μL (Mean ±SD)	7.812±4.2	9.455±4.6	0.127
Lymphocyte count 10^3 /μL (Mean ±SD)	1.176±0.6	1.425±0.6	0.129
Monocyte count 10^3 /μL (Mean ±SD)	0.581±0.4	0.940±0.6	0.001
Eosinophil count 10^3 /μL (Mean ±SD)	0.120±0.1	0.195±0.2	0.075
Basophil count 10^3 /μL (Mean ±SD)	0.068±0.1	0.090±0.1	0.551
Neutrophil to lymphocyte ratio (Mean ±SD)	12.953±25.2	7.961±5.1	0.380
Platelet to lymphocyte ratio (Mean ±SD)	333.729±495.6	219.786±193.2	0.315
WBC to MPV ratio (Mean ±SD)	0.889±0.4	0.973±0.3	0.442
MPV to lymphocyte ratio, (Mean ±SD)	16.224±19.9	10.447±4.8	0.202
Plateletcrit (%)	2.970±1.6	3.061±1.1	0.811
Creatinine (mg/dL)	1.150±0.6	2.086±1.7	0.001
High Sensitive Troponin I (ng/L)	65.921±50.8	183.895±133.5	0.001

Abbreviations: MCV, Mean corpuscular volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width; MPV, Mean Platelet Volume.

Discussion

This study focused on the role of haematological indices and their derivatives in patients with ACS and an apparently healthy non-ACS comparative group, which was the first to be performed in the study area. Baseline characteristics for both groups were analyzed: ACS was dominated by 74 males (67.3%), and the mean age was 56.69 ± 11.91 years, which is less than 60 years. This outcome is in line with the findings of a prior Makassar research.[13] In fact, compared to other regions like Europe, African countries had younger age morbidity and death from non-communicable diseases, mainly cardiovascular disease.[14] On the other hand, according to Dai et al, patients older than 65 account for almost 60% of ACS hospital beds.[15] The quick changes in epidemiology that have occurred in African nations could be the cause of this discrepancy.

In this study, WBC, MCHC, RDW%, RDW-SD, PLC, MPV, neutrophils, basophils, NLR, PLR, WBC-to-MPV ratio, MPV-to-lymphocyte ratio, troponin-I, and plateletcrit were significantly higher in the ACS group, whereas RBC, HGB, HCT, lymphocytes, and eosinophils were significantly higher in the non-ACS control groups (p <0.05). This outcome is consistent with earlier research that found a significant increase in the MCHC value in ACS patients as compared to healthy controls.[16] In contrast, a study conducted in India showed no substantial differences in RDW and HCT between patients with ACS and healthy controls.[17] According to Koorts AM. et al, patients with ACS have a complicated interplay affecting their red blood cell profile that involves inflammation, iron metabolism, and anemia. The body lowers serum iron levels during inflammation by regulating macrophages and duodenal absorption.[18] Our findings support the hypothesis that people diagnosed with acute MI have higher MPV. Cameron et al demonstrated that not only does mean platelet volume correlate with more ischemic events, but MPV also remained specifically high for several weeks after infarction.[19] Furthermore, Alvitigala et al found that, in comparison to the healthy control group, STEMI patients had considerably higher mean MPV and PDW.[20]

Troponin is generally known as a cardiac biomarker elevated during ischemia. Cardiac troponin levels begin to elevate approximately 2 or 3 hours after myocardial injury. Correlations between haematological indices and highly sensitive troponin-I, the gold standard for ACS diagnosis, were computed in both study groups separately. Accordingly, RDW%, RDW-SD, and MPV were significantly positively correlated with hs-Tn-I (r = 0.248, 0.189, and 0.245, respectively; $p < 0.05$), whereas there were no significant positive or negative correlations in the non-ACS control group. According to research by Lippi et al, the sensitivity of cardiac troponin levels for detecting ACS increased from 94% to 99% when cardiac troponin and RDW were measured together at admission.[21] According to a different study, RDW strongly predicted acute myocardial infarction in female patients.[22] RDW is a low-cost, easy-to-use laboratory measurement technique that can predict ACS with a reasonable degree of diagnostic accuracy. Baseline RDW assessment appears effective for predicting myocardial damage at an earlier time point, given that the unique kinetics of troponin in the injured myocardium restricts its utility within 2–4 hours of symptom onset.

Previous research has demonstrated that patients with ACS have a greater MPV, which is consistent with our findings.[23–25] Due to platelet metabolic and enzymatic activity, an elevated MPV is associated with a number of cardiovascular risks as well as increased thrombogenicity.[26,27] In contrast to our findings, a study done by Kevin Luke et al showed that patients with ACS had significantly lower MPV than those with SCAD.[28] When predicting ACS, RDW and MPV should be taken into account in addition to traditional cardiac indicators. They should also be used as a reference when choosing the best course of treatment.

In this study, haematological indices and biochemical markers were compared based on the outcomes. The mean differences in MPV, RDW, monocyte count, creatinine, and hs-Tn-I were substantially greater among non-survivors, whereas the mean corpuscular volume (MCV) was higher among ACS survivors. This result is consistent with prior research, as Shahin et al, found that patients who passed away from MI had substantially greater MPV.[29] Małyszczak et al showed that both low and high MPV are significantly associated with a higher 5-year mortality rate than normal MPV cases.[30] Consistent with our findings, Raised RDW is a valuable indicator for morbidity and death in heart failure patients, according to Felker's research.[31] According to Tonelli et al, mortality risk was correlated with greater RDW levels in those who had previously experienced MI but did not exhibit symptoms of heart failure.[32]

Furthermore, research demonstrated a link between an increased RDW and a greater death risk in myocardial infarction patients.[10,33–35] Circulating monocytes interact largely with endothelial cells and platelets, aggravating prothrombotic and inflammatory pathways, and serving as a source of numerous cytokines and chemicals.[36] We measured creatinine and hs-troponin concentrations, and, as expected, mean creatinine and hs-troponin levels were significantly higher in the non-survivor group. Our Results are consistent with earlier research, which showed that creatinine clearance is a significant independent predictor of severe bleeding and in-hospital death in ACS patients.[37–39] Our study showed rising hs-Troponin I levels among non-survivors of ACS. Since levels of troponin and injured myocardial mass are strongly correlated, patients with ACS typically have greater troponin levels. Increased troponin levels are also an indicator of poor outcomes.[40] According to Kanani et al, greater hs-Troponin I readings were significantly linked to worse outcomes in the emergency room for both sexes; however, females had a higher inpatient mortality rate.[41]

Strengths and Limitations

To highlight the strength of this study, we extracted various types of information, such as cardiac parameters, hematologic indices, and biochemical markers. We examined how these variables were related to the clinical diagnoses and outcomes.

It is important to acknowledge the limitations of our investigation. Firstly, the study was cross-sectional, meaning that we may require additional time to observe and monitor changes and their impacts on the outcome. Additionally, the study involved a relatively small group of patients. To draw more conclusive results, we recommend a prospective cohort study with extended follow-up and a larger sample size. Such study should encompass a thorough analysis of cardiac, hematologic, and metabolic parameters, as well as consider various treatment modalities for patients with ACS.

Conclusion

This study explored the haematological profiles and their association with clinical outcomes in patients with ACS compared to healthy controls. Significant differences in haematological indices were observed between the groups, highlighting the potential of these markers in ACS diagnosis and prognosis. Correlations with highly sensitive troponin-I further emphasized the diagnostic

utility of parameters like RDW and MPV. Notably, differences in these markers also reflected outcomes, with non-survivors exhibiting distinct profiles. These findings underscore the importance of haematological indices in ACS management and prognosis, offering valuable insights for clinical practice and future research directions.

Abbreviations

ACS, Acute Coronary Syndrome; AMI, Acute Myocardial Infarction; GRACE, Global Registry of Acute Coronary Events; NSTEMI, Non-ST Elevation Myocardial Infarction; STEMI, ST Elevation Myocardial Infarction.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because it contain information that could compromise the privacy of research participants.

Ethical Approval and Informed Consents

The Jimma University Institutional Research Board (IRB) reviewed and approved the study protocol in accordance with the Declaration of Helsinki, assigning it the number IHRPGD/554/2022. Participants in the study were informed of the purpose of the investigation and the importance of their participation (or, in the event that the patient is unable of communicating or providing consent, their family member or caregiver). Written informed consent was obtained from each participant prior to any data collection. We certify that this article conforms to all applicable local, state, federal, and international laws pertaining to consent and privacy.

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Disclosure

The authors report no conflicts of interest in this work.

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CASE REPORT

Successful Treatment of Steroid-Refractory Immune Thrombocytopenia in a Patient Developing Multiple Myeloma While on Immune Checkpoint Inhibitor Therapy for Lung Cancer: A Case Report

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Abstract: Immune checkpoint inhibitor-related thrombocytopenia (irTCP) is a relatively rare immune-related adverse event (irAE); however, overall survival may worsen when it occurs. Prolonged use of high-dose steroids can diminish the effectiveness of immune checkpoint inhibitor (ICI) therapy on the primary disease because of T lymphocyte suppression, thus early tapering is necessary. We experienced a rare case of a 79-year-old male who concurrently developed irTCP and multiple myeloma (MM) during treatment with ICIs for lung adenocarcinoma. The patient exhibited severe thrombocytopenia and elevated serum IgA levels. Based on various tests, we diagnosed MM and irTCP. Despite administering the standard bortezomib plus dexamethasone (Bd therapy) treatment for MM, there was no response and the irTCP was steroid-resistant. Consequently, we administered a regimen including daratumumab (DPd therapy) for steroid-resistant irTCP and refractory MM, which resulted in a response. As a result, we were able to avoid prolonged use of high-dose steroids and the patient is stable without exacerbation of lung adenocarcinoma for 1 year and 5 months after the onset of MM. To our knowledge, there are no cases of MM developing during ICI treatment and this is the first case report in which daratumumab was effective for the treatment of irTCP.

Keywords: immunotherapy, immune-related adverse event, multiple primary cancer, immune-mediated hematologic toxicity, monoclonal antibody

Introduction

Immune checkpoint inhibitors (ICIs) are becoming a standard treatment for various cancers, such as malignant melanoma, lung cancer, gastric cancer, and colorectal cancer. Consequently, various side effects known as immune-related adverse events (irAEs), such as diabetes, colitis, and myocarditis, have been identified. The importance of managing these side effects has become increasingly recognized.[1,2] Immune checkpoint inhibitor-related thrombocytopenia (irTCP) is relatively rare among irAEs.[2] For differentiating thrombocytopenia during cancer treatment, numerous factors must be considered. These include 1) drug-induced thrombocytopenia associated with chemotherapy including ICIs, 2) hemophagocytic syndrome caused by ICIs,[3,4] 3) disseminated intravascular coagulation or marrow carcinosis resulting from cancer, 4) hematologic disorders, such as secondary myelodysplastic syndromes, 5) anti-HLA antibodies resulting from transfusions, and 6) liver diseases, such as cirrhosis or liver failure. Therefore, diagnosing irTCP is often more challenging compared with immune thrombocytopenia (ITP). The coexistence of irTCP may result in a worsening of overall survival (OS).[5] Factors contributing to the deterioration of OS include severe bleeding due to thrombocytopenia, limited treatment options for primary disease due to the impact of irAEs, including irTCP, and the inability to resume chemotherapy because of a poor response to irTCP treatment. Severe cases have resulted in fatalities,[6] and reports suggest that irTCP has a higher risk of reaching grades 3–4

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compared with other hematologic irAEs.[7] Moreover, there is no evidence indicating that ICIs cause secondary cancers, including hematologic diseases, and there are no established treatment protocols for complications arising from the coexistence of irTCP and secondary cancers. In the present case, during the treatment of lung adenocarcinoma with ICIs, the patient developed multiple myeloma (MM) and irTCP. We successfully managed the disease and its complications with daratumumab, which indicates that this approach represents a new treatment strategy.

Case Presentation

A 79-year-old Asian male was diagnosed with lung adenocarcinoma stage cT3N2M1a in August 2021. At diagnosis, CYFRA was 1.3 ng/mL (normal upper limit: 3.5 ng/mL) and SCC was 1.8 ng/mL (normal upper limit: 2.0 ng/mL). First-line treatment was initiated with nivolumab plus ipilimumab (Nivo + IPI) and the patient has since maintained a partial response. In April 2022, after the 11th course of Nivo + IPI, the patient developed adverse events with a white blood cell count of 1,600/μL (Grade 3), a neutrophil count of 550/μL (Grade 3), and a platelet count of 50,000/μL (Grade 2), resulting in the discontinuation of chemotherapy; however, the cytopenia progressed even after the postponement of Nivo + IPI treatment. The patient had a creatinine level of 2.42 mg/dL, indicating renal impairment, total protein of 9.6 g/dL, albumin of 2.6 g/dL, indicating total protein-albumin dissociation, and elevated serum immunoglobulins with IgA at 4,469 mg/dL (normal range: 93–393 mg/dL), IgG at 983 mg/dL (normal range: 861–1,747 mg/dL), and IgM at 134 mg/dL (normal range: 33–183 mg/dL). Physically, the patient presented with anemic conjunctiva and pain in both rib areas. Immunoelectrophoresis revealed M-protein. Bone marrow examination showed a nucleated cell count of 8.5×10^4 μ/L (normal range: 10–25 × 10^4 μ/L), megakaryocyte count of 51.0 μ/L (normal range: 50.0–15.0 μ/L), plasma cells at 10.0% (normal range: 0.2–2.0%) (Figure 1), and megakaryocytes at 0.4% (normal

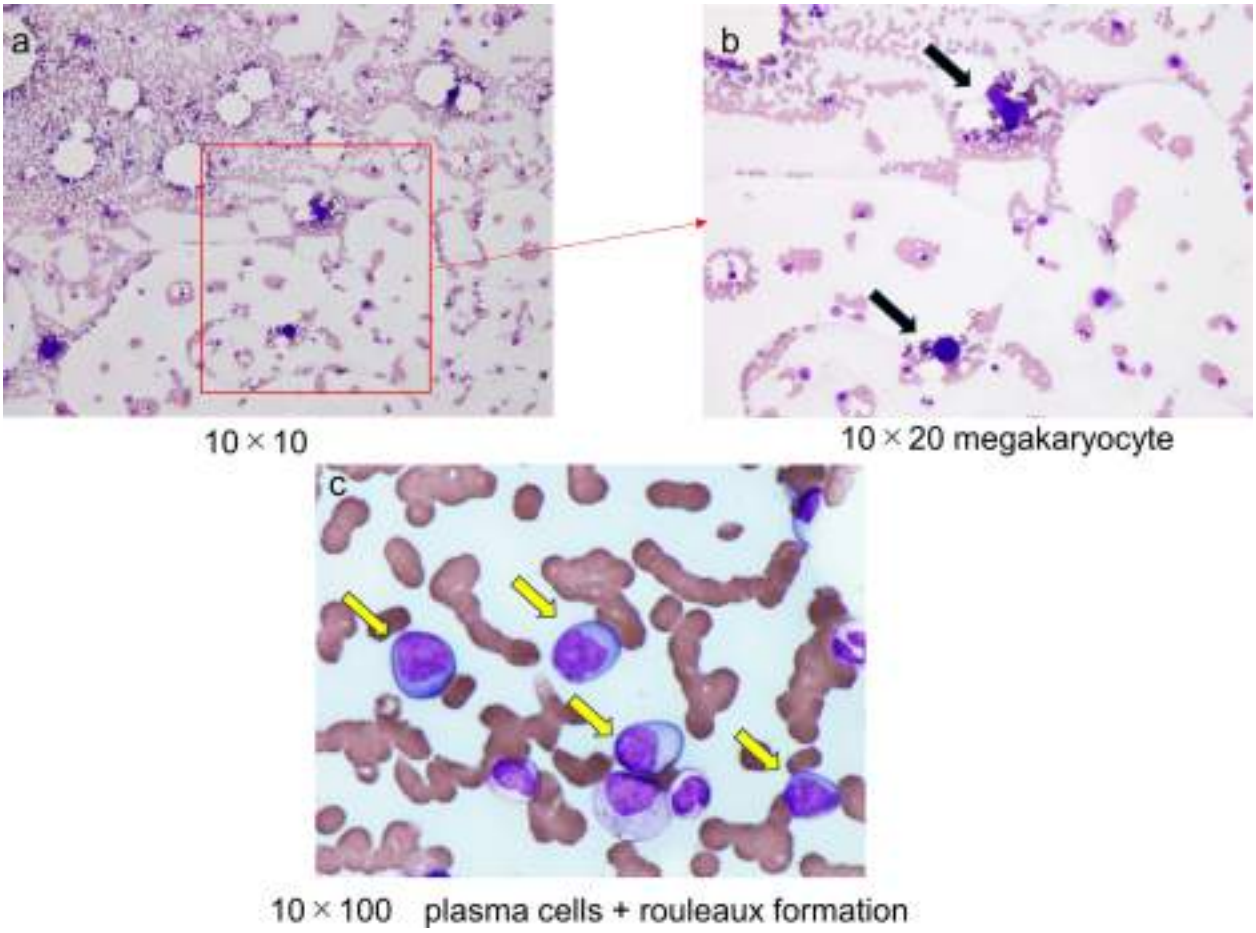

Figure 1 The bone marrow aspiration. (**a** and **b**) The bone marrow is hyperplastic, with an increase in megakaryocytes. Cells at various stages of maturation in all three lineages are observed. No obvious atypical epithelial cells are noted. (**c**) There is an increase in plasma cells.

range: 0–0.2%). Flow cytometry revealed a profile of CD19- CD20- CD38+ CD56+ CD138+ cyλ. Magnetic resonance imaging (MRI) revealed abnormal signals and postcontrast enhancement, predominantly in the vicinity of the left jugular foramen, near the sternum of the right middle cranial fossa, and at the anterior arch of the C1 cervical spine at the base of the skull. Bone scintigraphy revealed heterogeneous abnormal uptake in the sternum, cervical-thoracic-lumbar-sacral vertebrae, left and right ilia, and right ischium, along with multiple uptakes in the left and right ribs (Figure 2). MRI showed bone lesions in the head, and bone scintigraphy revealed accumulations in the ribs and spine, confirming bone lesions in two or more locations in MM. The patient was diagnosed with MM based on the presence of 10% plasmacytes in the bone marrow showing clonality by FCM, IgA M-protein (4,469 mg/dL) and three myeloma defining events; 1) renal impairment (CrCL 24.8 mL/min < 40 mL/min, serum creatine 2.42 mg/dL > 2 mg/dL), 2) anemia (hemoglobin 9.3 mg/dL < 10 mg/dL) and 3) hypercalcemia (serum calcium 12.5 mg/dL > 11 mg/dL), along with myeloma defining biomarkers; the presence of 2 or more bone lesions measuring 5 mm or more in size on MRI study. β2-microglobulin was 13.8 mg/L and LDH was 244 U/L. FISH was negative for P53 deletion, IgH-FGFR translocation, and IgH-MAF translocation. The diagnosis was IgA λ-type MM, classified as R-ISS Stage III. With respect to thrombocytopenia, the bone marrow megakaryocyte count was 51.0 μ/L (normal range: 50.0–15.0 μ/L) and the immature platelet fraction (IPF) was high at 6.8%, suggesting that the thrombocytopenia was not attributable to MM. Pathological examination of the bone marrow clot revealed all three blood cell lineages and no overt atypical epithelial cells, negating the possibility of thrombocytopenia resulting from bone marrow carcinosis of lung adenocarcinoma. TSH was 2.82 μIU/mL, FT4 was 1.16 ng/dL, with no thyroid dysfunction. Thrombocytopenia resulting from disseminated intravascular coagulation or other drug-induced causes was ruled out. Instead, thrombocytopenia in this case was diagnosed as irTCP. Computed tomography scan showed maintenance of shrinkage in the primary lesion of the lung with obliteration of the right middle lobe and multiple right pleural metastatic layers. There was no significant change in the size of the bilateral hilar and mediastinal lymph nodes, or the multiple lymph nodes in the right supraclavicular fossa, thus maintaining stable disease.

To prioritize the treatment of MM, first-line therapy was initiated with bortezomib (BOR 1.3 mg/m2 on days 1, 8, 15, 22) plus dexamethasone (oral DEX 20 mg on days 1, 8, 15, 22), which is referred to as Bd therapy; however, posttreatment, high serum IgA levels maintained, and bone marrow examination revealed a poor response. Furthermore, the steroid component of the therapy was ineffective against irTCP, resulting in a dependence on transfusions.

To treat refractory MM, the regimen was switched to daratumumab (Dara 1800 mg on days 1, 8, 15, 22) + pomalidomide (Pom 2 mg daily on days 1–21) + dexamethasone (DEX 16.5 mg on days 1, 8, 15, 22), which is known as DPd therapy. This resulted in an improvement in IgA levels (Figure 3a), indicating a response to MM. At the time of diagnosis, the creatinine level

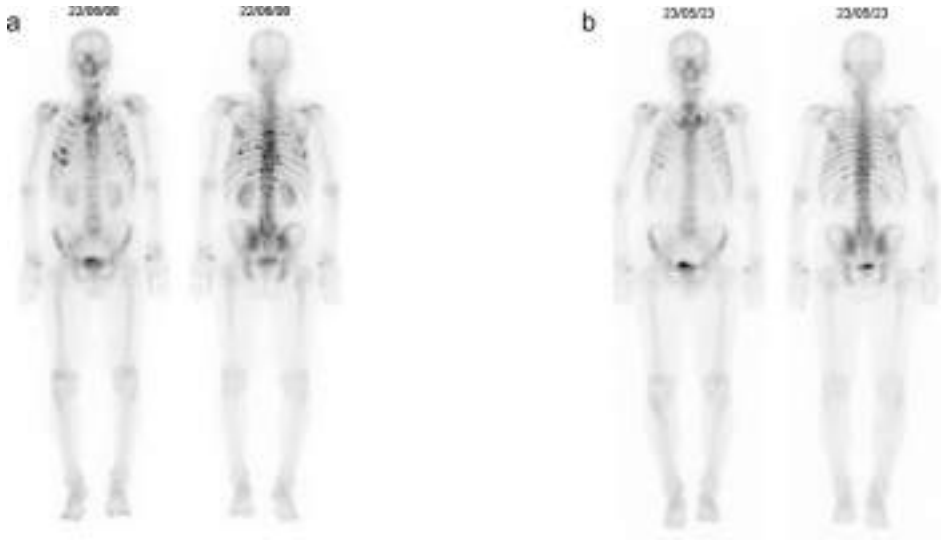

Figure 2 Bone scintigraphy (**a**) At the time of MM diagnosis. Abnormal accumulations are noted in the right sternum, left temporal bone, sternum, cervical-thoracic-lumbar-sacral vertebrae, left and right ilia, and right ischium. (**b**) After the completion of nine courses of DPd therapy. 1 year after the initiation of MM treatment. Although some residual accumulations are noted, the multiple abnormal accumulations observed previously have improved, suggesting that they were associated with pathological fractures caused by MM, rather than MM lesions themselves.

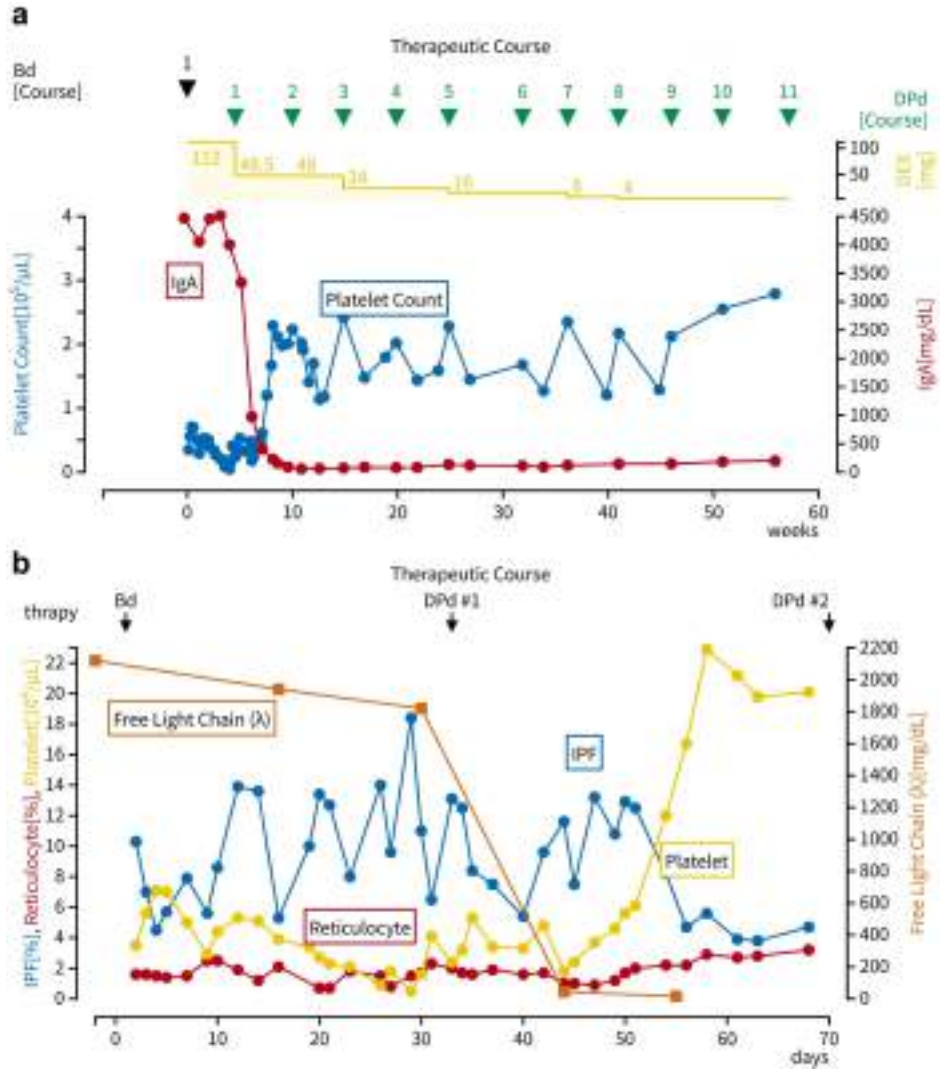

Figure 3 Therapeutic course. (**a**) Created with September 9th, the start date of the first course of Bd therapy, as day 1. (**b**) Before DPd treatment, reticulocyte generally remained within the normal range of 0.7–2.3% (normal range: 0.8–2.0%), with no decline observed, and the IPF has consistently remained high. After DPd treatment, a decrease in IPF and an increase in platelet count were observed.

was 2.42 mg/dL, which later increased to 4.18 mg/dL. However, after DPd therapy, the creatinine level improved significantly to 0.70 mg/dL, suggesting the presence of myeloma kidney. In addition, following DPd therapy, a decrease in IPF and an increase in platelet count were observed, which eliminated the need for transfusions (Figure 3b). After achieving a response with DPd therapy for MM, steroid administration was rapidly reduced early in the course of treatment. As of August 2023, the patient had undergone 12 cycles of outpatient DPd therapy. Computed tomography scans revealed that shrinkage was maintained in the primary lesion of the right middle lobe obliteration and multiple right pleural metastatic layers in the lung. There was also no significant change in the size of the bilateral hilar and mediastinal lymph nodes, or the multiple lymph nodes in the right supraclavicular fossa, indicating a progression-free status. Furthermore, the accumulations in the ribs and spine observed in the bone scintigraphy before treatment were improved one year after the start of treatment, suggesting through clinical progression that they were associated with pathological fractures caused by MM, rather than MM lesions themselves (Figure 2).

Discussion

Thrombocytopenia can occur as an irAE; however, it is rare.[2] The coexistence of irTCP may worsen OS.[5] To diagnose irTCP, it is essential to differentiate thrombocytopenia during ICI therapy by ruling out other causes, such as bone

marrow infiltration by cancer, hematologic diseases including secondary myelodysplastic syndromes or acute myeloid leukemia, and other drug-induced effects, which make diagnosis challenging.[8] Although there are cases of concurrent lung cancer and MM,[9] to our knowledge, there have been no examples of another cancer developing during ICI treatment. Therefore, it is unclear whether the MM was secondary to the ICI therapy or occurred coincidentally. The case was further complicated in its diagnosis of irTCP because of the severe thrombocytopenia and concurrent MM during ICI therapy for lung adenocarcinoma.

irTCP and ITP are thought to have similar pathologies, suggesting the production of anti-platelet antibodies.[10,11] Therefore, steroid therapy was considered effective. However, in this case, there was no response to dexamethasone, leading to a dependence on platelet transfusions. Switching to DPd therapy resulted in a response to both the failure of induction therapy in MM and steroid-resistant irTCP. Before DPd treatment, reticulocyte generally remained within the normal range of 0.7–2.3% (normal range: 0.8–2.0%), with no decline observed. Thus, we concluded that there was no bone marrow suppression due to MM. Furthermore, if it were suppression from MM, the IPF should be low; however, the IPF has consistently remained high, and following DPd therapy, a decrease in IPF and an increase in platelet count were observed. It is difficult to distinguish between ITP caused by MM and irTCP. Instances of ITP related to MM are rare,[12] and the concurrent diagnosis of both conditions is even less common.[13] Interestingly, in cases where ITP is linked to MM, the M-protein is typically IgG.[13] In this case, ITP due to MM could not be excluded as a potential cause of the patient's thrombocytopenia. However, because the patient had previously undergone ICI treatment, was diagnosed with MM concurrently, and the M-protein was IgA, we determined that the thrombocytopenia was attributed to irTCP based on the course of the disease. To date, there have been no reports of successfully treating thrombocytopenia with pomalidomide caused by irTCP, suggesting that the effect may be attributed to daratumumab in the DPd regimen. Rituximab, which is indicated for refractory ITP, works by suppressing the production of anti-platelet antibodies from activated B cells.[14,15] Daratumumab is a monoclonal antibody that targets CD38,[16] an antigen expressed on normal activated T and B cells, NK cells, monocytes, and plasma cells. It is hypothesized that daratumumab improves irTCP, not only by acting on plasma cells, but also on activated B cells, thereby ameliorating the exacerbation of platelet destruction caused by autoantibody production.

Steroids are the standard treatment for irTCP, but they are not always effective against severe thrombocytopenia (grades 3–4). Furthermore, there are reports that irTCP can reduce OS.[5] Considering reports of decreased survival with high-dose steroid administration for irAEs,[17] this may be considered a contributing factor. This suggests that the worsening of OS is not only due to the severity of irAEs, which require high doses of steroids, but the use of steroids may have adversely affected the primary disease by suppressing the effect of ICIs on T lymphocytes. Indeed, there are reports of successful treatment with thrombopoietin drugs for steroid-resistant thrombocytopenia, following the initiation of pembrolizumab.[18] Guidelines recommend the use of IVIg, rituximab, or thrombopoietin agents for cases, in which thrombocytopenia as an irAE develops and the response to steroid therapy is inadequate.[19,20] In this case, daratumumab may be added to standard Bd therapy for refractory MM to successfully treat both MM and steroid-resistant irTCP. Furthermore, it was possible to reduce high-dose steroid dosages without long-term use. As a result, there was no worsening of the lung adenocarcinoma 1 year and 5 months after the onset of MM. We hypothesized that daratumumab may have less impact on T lymphocytes compared with high-dose steroids, thus sustaining the effectiveness of the ICIs. As the indications for ICIs expand, cases of irAEs, including irTCP, in combination with other cancer types, will likely increase. We demonstrated the importance of early management of irAEs, considering efficacy for the concurrent cancer (MM), and of maintaining awareness of treatment effectiveness for the target cancer (lung adenocarcinoma).

Conclusion

We encountered a rare case of concurrent MM and irTCP during ICI therapy for lung adenocarcinoma. By focusing on the common mechanisms of action in irTCP and MM, we successfully achieved a response with daratumumab. During the development of secondary cancers during ICI administration, it is necessary to consider not only the concurrent cancer, but also the treatment of the target cancer. Therefore, it is necessary to reduce steroid therapy promptly if a response is achieved for irTCP to avoid decreasing the efficacy of ICIs on the target cancer.

Ethics and Consent

The patient provided written informed consent for publication of the case report. Institutional approval was not required to publish the case details.

Disclosure

The authors report no conflicts of interest in this work.

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CASE REPORT

Clinical and Genomic Profile of Primary Cranial Neurolymphomatosis

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Abstract: Primary cranial neurolymphomatosis (PCNL) is a rare subtype of primary CNS lymphoma (PCNSL) in which infiltrative lymphomatous involvement is confined to cranial nerves. Here, we report a case of PCNL with successful genomic profiling. A 57-year-old male had a lengthy prediagnostic phase spanning approximately 30 months, characterized by multiple episodes of cranial neuropathies managed by steroids. At the time of diagnosis, the patient had right-sided cranial neuropathies involving cranial nerves (CN) V, VI, and VII. Pathological findings of the right cavernous lesion biopsy were consistent with large B-cell lymphoma-infiltrating nerve fibers. The clinical course was aggressive and refractory, characterized by relentless progression with the development of cervical spinal neurolymphomatosis, cerebrospinal fluid involvement, and ependymal and intraparenchymal cerebral involvement, despite multiple lines of therapy, including chemoimmunotherapy, Bruton's tyrosine kinase inhibitor, radiation, autologous stem cell transplant, chimeric antigen receptor T-cell therapy (CAR-T), and whole-brain radiation. The patient survived for 22 months from the time of the initial diagnosis and 52 months after the first episode of cranial neuropathy. Next-generation sequencing identified mutations (MYD88, CD79b, and PIM1) that are frequently observed in PCNSL. The unusual findings included a total of 22 mutations involving PIM1, indicating a highly active aberrant somatic hypermutation and two missense CXCR4 mutations. CXCR4 mutations have never been described in PCNSL and may have implications for disease biology and therapeutic interventions. We provide a literature review to further elucidate PCNL.

Keywords: DLBCL, cranial nerve, autologous stem cell transplant, CAR-T cell therapy, next generation sequencing

Introduction

Neurolymphomatosis (NL) refers to infiltrative lymphomatous involvement of the cranial, spinal, and peripheral nerves and nerve plexuses and is the least common neurological presentation of lymphoma.[1,2] Neuropathy resulting from compression or entrapment by adjacent large lymphomatous tumors is not considered NL. It is a rare lymphoma entity, as revealed by the largest retrospective studies from large institutions (Mayo Clinic and Massachusetts General Hospital) and the International Primary CNS Lymphoma Collaborative Group (IPCG) in which 40, 25, and 50 cases were gathered over 16, 28, and 15 years respectively.[1–3]

Based on these large studies, the median age at diagnosis of NL is approximately 55.5–63 years with a slight male preponderance of 54–60%. Most cases were B-cell non-Hodgkin lymphoma (NHL) (82–97%). Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma associated with NL (68–75.5%).[1–3] NL is divided into primary and secondary NL depending on the absence or presence of systemic lymphoma. Clinical manifestations depend on the type of nerve involved: cranial nerves, spinal nerves, cauda equina, peripheral nerves, or nerve plexuses. The neurological symptoms can be sensory, motor, or mixed.

Cranial nerve involvement in DLBCL has been regarded as central nervous system (CNS) involvement.[4] Thus, primary cranial nerve NL is a subtype of PCNSL in which lymphomatous involvement is confined to cranial nerves. However, this condition is extremely rare, and the biology, clinical course, and natural history have not been well studied.

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Here, we describe the clinical course of a 57-year-old patient with PCNL, the genomic profile of the tumor, and a literature review.

Case Presentation

A 57-year-old male patient presented to our institution for the evaluation of multiple cranial neuropathies, including bilateral cranial nerve (CN) VII and right CNs III, V, and VI, spanning the previous 30 months. The outside magnetic resonance imaging (MRI) brain showed some enhancement of the cranial nerves, but cerebrospinal fluid (CSF) analysis was negative. He was initially managed with steroids and azathioprine was subsequently administered. His neurological symptoms improved transiently with these treatments but recurred with a reduction in the steroid dose.

At the time of evaluation, he experienced right-sided facial weakness, diplopia, and pain. Positive findings on neurologic examination included ophthalmoplegia of the right eye with limited abduction and adduction, decreased sensation of soft touch in the right V1-V2 distribution, and right-sided facial droop with weakness of the right eye closure and inability to puff the right cheek. The neurological findings were consistent with palsies of the right CN V, VI, and VII. The patient underwent an extensive diagnostic evaluation.

Magnetic resonance imaging (MRI) of the brain revealed asymmetric prominent enhancement of the right seventh cranial nerve at the mastoid segment, extending into the right parotid space, and asymmetric thickening and enhancement of the right cavernous sinus and right foramen rotundum (Figure 1). No significant abnormalities were observed in the brain parenchyma or spinal cord. Positron emission tomography (PET) scan utilizing fludeoxyglucose (FDG) radiotracer showed a pancreatic lesion, and biopsy revealed a low-grade neuroendocrine tumor. The abnormalities found on MRI involving the brain were not seen on PET scan. The CSF analysis results were negative. He underwent a right craniotomy and biopsy of the enhancing right cavernous sinus lesion, which was consistent with a large B-cell lymphoma involving nerve fibers (Figure 2). Pathology revealed multiple

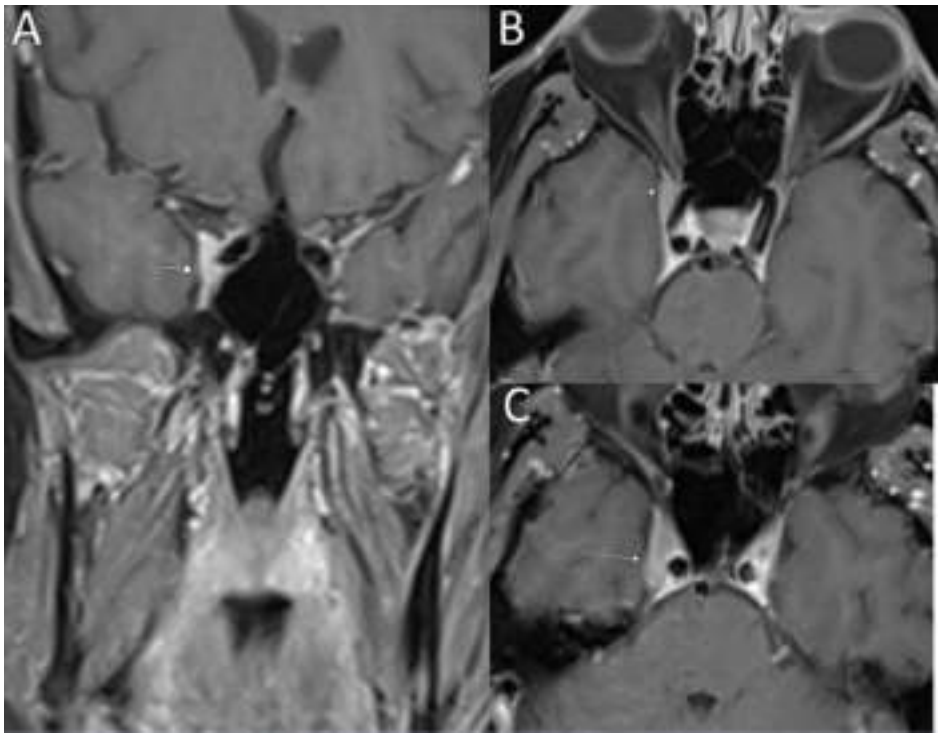

Figure 1 57-year-old man with multiple cranial neuropathies. Coronal (**A**) and axial (**B** and **C**) contrast-enhanced T1-weighted MR images showing a nodular enhancing lesion within the anterior right cavernous sinus (arrows) (B-cell lymphoma on biopsy).

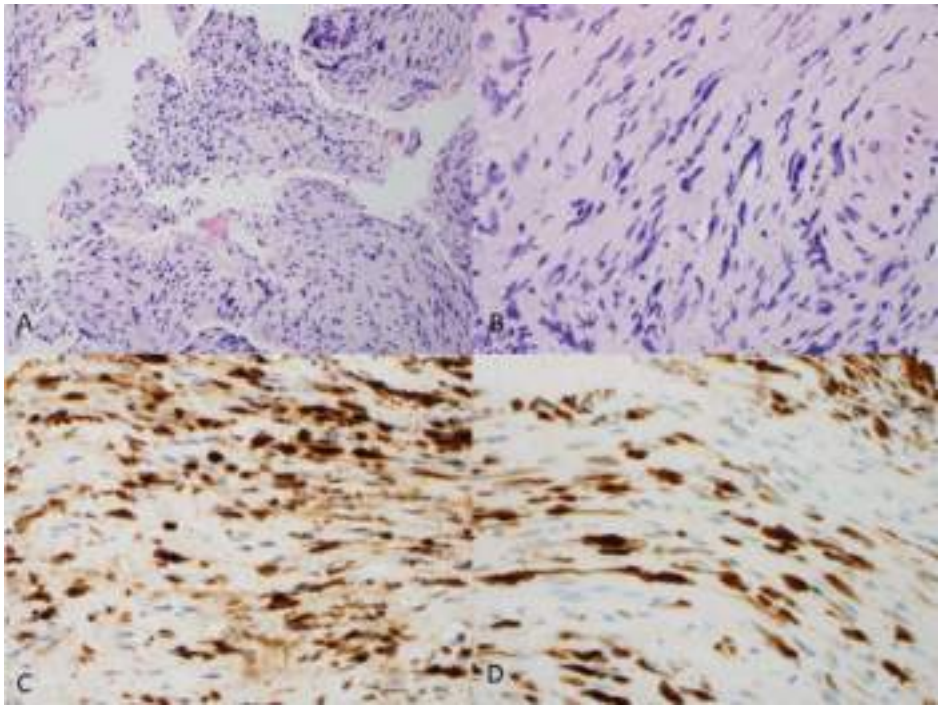

Figure 2 Biopsy of the right cavernous sinus lesion. The biopsy of the right cavernous sinus lesion comprises multiple fragments of nerve with the diffuse interstitial infiltrate of large, atypical cells (**A** and **B**, x 20 and x 40; respectively). The large, atypical cells are diffusely positive for PAX-5 (**C**, x 40) and CD20. The proliferative rate by Ki-67 is high (80%) (**D**, x 40). The findings confirmed a high-grade B cell lymphoma involving the cranial nerve.

fragments of nerves diffusely infiltrated by CD20+ and PAX5+ large atypical cells, with a Ki-67 proliferation index of 80%. A pathological diagnosis of cranial large B-cell neurolymphomatosis was made.

Formalin-fixed paraffin-embedded lymphoma tissue was sent for comprehensive genetic profiling using Hematology Profile Plus by the Genomic Testing Cooperative. Hematology Profile Plus uses next-generation sequencing, Sanger Sequencing, and fragment length analysis to identify molecular abnormalities in the DNA of 179 genes and RNA of 1408 genes associated with hematologic malignancies. The results are presented in Table 1. These findings are consistent with the activated B-cell (non-germinal center phenotype) signature with mutations in MYD88, CD79b, PIM1, CXCR4, and IKZF3.

Table 1 Genomic Profiling of the Tumor

Genomic Alterations				
Gene Name	Hgvsp	Consequence	Allele Frequency (%)	Predicted Effect
CD79B	NP_001035022.1:p.Tyr197Cys	Missense	24.03	Deleterious
CD79B	NP_001035022.1:p.Asp194Ala	Missense	23.27	Deleterious
CDC73	NP_078805.3:p.Ile252Met	Missense	20.61	Tolerated
CXCR4	NP_001008540.1:p.Arg152Gly	Missense	23.23	Deleterious
CXCR4	NP_001008540.1:p.Ile226Phe	Missense	16.24	Deleterious

(*Continued*)

Table 1 (Continued).

IKZF3	NP_036613.2:p.Phe146Leu	Missense	16.85	Deleterious
MITF	NP_937802.1:p.Glu419Lys	Missense	48.32	Deleterious
MYD88	NP_001166038.1:p.Leu265Pro	Missense	12.0	Deleterious
PIM1	NP_001230115.1:p.Gln128His	Missense	36.48	Deleterious
PIM1	NP_001230115.1:p.Glu226Lys	Missense	25.88	Deleterious
PIM1	NP_001230115.1:p.Gly119Asp	Missense, Splice region variant	22.06	Deleterious
PIM1	NP_001230115.1:p.Trp200Ter	Stop gain	20.58	Deleterious
PIM1	NP_001230115.1:p.Lys115Asn	Missense	16.67	Deleterious
PIM1	NP_001230115.1:p.Gln218Ter	Stop gain	16.23	Deleterious
PIM1	NP_001230115.1:p.Leu273Arg	Missense	16.17	Deleterious
PIM1	NP_001230115.1:p.Ser188Ile	Missense	16.15	Deleterious
PIM1	NP_001230115.1:p.Arg227Met	Missense	15.88	Deleterious
PIM1	NP_001230115.1:p.Gly136Asp	Missense	15.69	Deleterious
PIM1	NP_001230115.1:p.Glu121Asp	Missense	14.8	Deleterious
PIM1	NP_001230115.1:p.His159Asp	Missense	14.03	Deleterious
PIM1	NP_001230115.1:p.Leu268Val	Missense	13.41	Deleterious
PIM1	NP_001230115.1:p.Gly169Arg	Missense	12.86	Deleterious
PIM1	NP_001230115.1:p.Ser166Ala	Missense	12.72	Deleterious
PIM1	NP_001230115.1:p.Cys252Tyr	Missense	10.19	Deleterious
PIM1	NP_001230115.1:p.Ser65Arg	Missense	9.25	Deleterious

(*Continued*)

Table 1 (Continued).

PIM1	NP_001230115.1:p.Asn251Lys	Missense	8.97	Deleterious
PIM1	NP_001230115.1:p.Leu116Val	Missense	7.86	Deleterious
PIM1	NP_001230115.1:p.Gly73Asp	Missense	7.61	Deleterious
PIM1	NP_001230115.1:p.Met92Ile	Missense	6.61	Deleterious
PIM1	NP_001230115.1:p.Ser77Arg	Missense	6.42	Deleterious
Heterogeneity				
	There are abnormal clones with PIM1 (22 mutations), CD79B (2 mutations), CXCR4 (2 mutations), CDC73, IKZF3, and MYD88 mutations. The MITF mutation is detected at a high level, possible germline abnormality.			
Expression Profile				
	Increased B-cell markers. No significant increase in BCL1, BCL2, or MYC mRNA			
Chromosomal Structural Analysis				
	3p-, 6q-, 8p+, and +12.			
Cell of Origin				
	Expression profiling suggests ABC cell of origin, but less aggressive subtype (ABC1).			

The initial induction therapy used was the RMA regimen: rituximab, high-dose methotrexate (HD-MTX), and high-dose ara-c (HiDAC). The patient had an excellent clinical response, with resolution of the neurological symptoms after the first cycle. Unfortunately, he developed acute renal failure related to HD-MTX following the second cycle, which required hemodialysis. The patient's renal function eventually completely recovered. Repeat MRI after 2 cycles of chemotherapy showed persistent enhancement around the right trigeminal and facial nerves. He was then treated with skull-base radiation therapy, followed by the initiation of ibrutinib (560 mg daily). After radiation and four months of ibrutinib treatment, MRI showed improved enhancement around the cranial nerves, with no new lesions. He underwent high-dose chemotherapy with carmustine (BCNU) and thiotepa, followed by autologous stem cell transplantation (ASCT).

He was found to have relapse/progression four months after ASCT, with MRI showing new abnormal enhancement involving the cisternal segments of bilateral CN III and V with new enhancements within the left internal auditory canal along CN VII and VIII. Within a month, he experienced rapid neurological progression with severe bilateral shoulder pain, new left facial palsy, and difficulty with mastication. MRI of the cervical spine also showed multilevel cervical nerve root enhancement (Figure 3), whereas MRI of the lumbar spine showed enhancement of the cauda equina. The CSF cytology was positive for lymphoma. The patient underwent cervical spinal radiation with symptomatic relief. He received one cycle of rituximab, ibrutinib, and lenalidomide. Owing to rapid clinical deterioration, he was switched to two cycles of R-EPOCH (rituximab, etoposide, vincristine, cyclophosphamide, and doxorubicin) in combination with intrathecal triple chemotherapy with methotrexate, ara-c, and hydrocortisone. Due to relapsed and chemorefractory disease, the patient was referred for chimeric antigen receptor (CAR) T-cell therapy (CAR-T). He

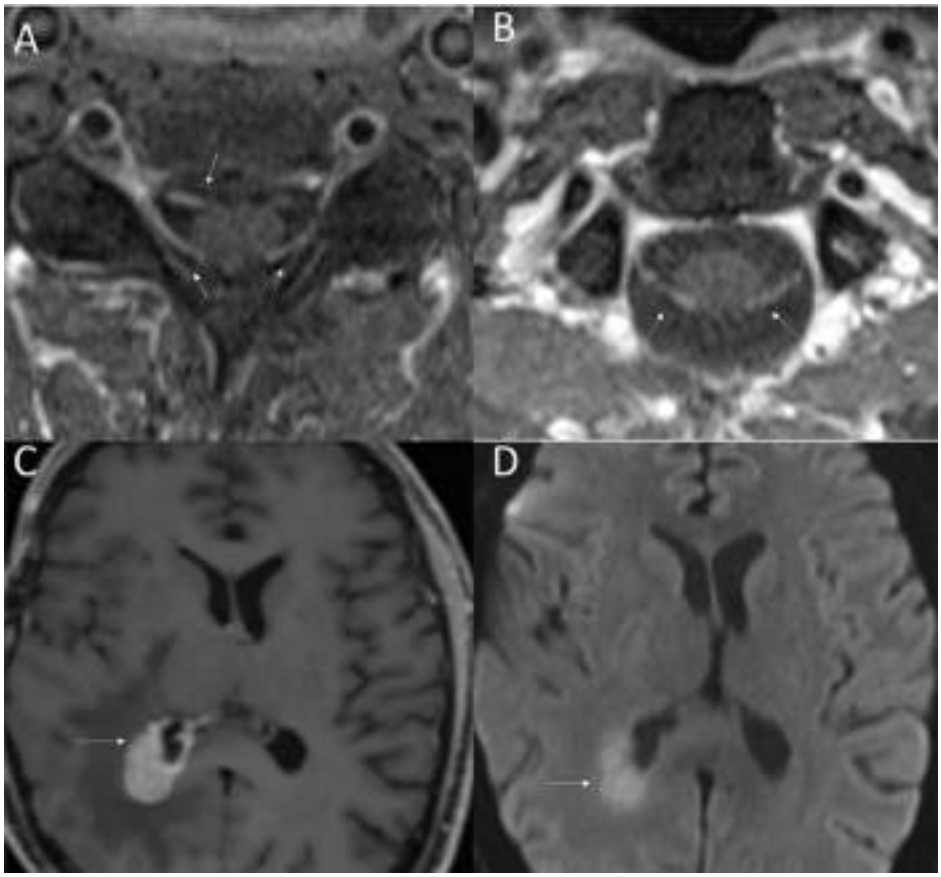

Figure 3 Follow up MRI of the cervical spine (**A** and **B**) and brain (**C** and **D**) after 1-year of initial presentation with progression of neurolymphomatosis. Axial (**A** and **B**) contrast-enhanced T1-weighted MR images of the cervical spine with thickening and enhancement of multiple dorsal and ventral nerve roots. Contrast-enhanced T1-weighted MRI of the brain reveals nodular enhancing lesion with ependymal involvement in the right peritrigonal region (**C**, arrow). Lesion shows restricted diffusion (**D**, arrow) suggesting high cellularity.

received low-dose fludarabine and cyclophosphamide lymphodepleting therapy followed by tisagenlecleucel infusion. CAR-T cell therapy was complicated by grade 1 cytokine release syndrome (CRS). MRIs performed 30 days post-CAR-T cell therapy showed interval improvement in the cranial nerve and cervical spine enhancement. However, MRI three months post-CAR-T showed disease progression with leptomeningeal and ependymal involvement (Figure 3). The patient returned home and underwent modified whole-brain radiation, excluding the skull base and upper cervical spine, based on prior treatments. Three months later, his disease progressed rapidly, with an outside MRI report indicating disseminated meningeal, ependymal, and brain parenchymal involvement, leading to his death 22 months after his initial diagnosis and 52 months after the initial episode of cranial neuropathy. The clinical timeline is shown in Figure 4. Throughout the disease course, he had periodic CT imaging that did not identify any lymphomatous involvement outside of the CNS. Additionally, monitoring of his pancreatic low-grade neuroendocrine tumor showed stability, and he never required treatment for this lesion.

Discussion

Our case illustrates many unique features that can be seen in PCNL. The patient had a rather long pre-diagnostic phase, spanning approximately 30 months, during which he had multiple relapsing episodes of cranial neuropathies. The patient was managed with steroids, which alleviated the symptoms; however, he was never in remission. Although there was no definitive evidence of PCNL during these months, it is quite likely that the patient already had PCNL. An analogous clinical scenario has been observed in PCNSL, in which some patients have control or even remission of the disease with steroid treatment only.[5] The PCNL in our patient shares this feature of steroid responsiveness, as described in PCNSL. As

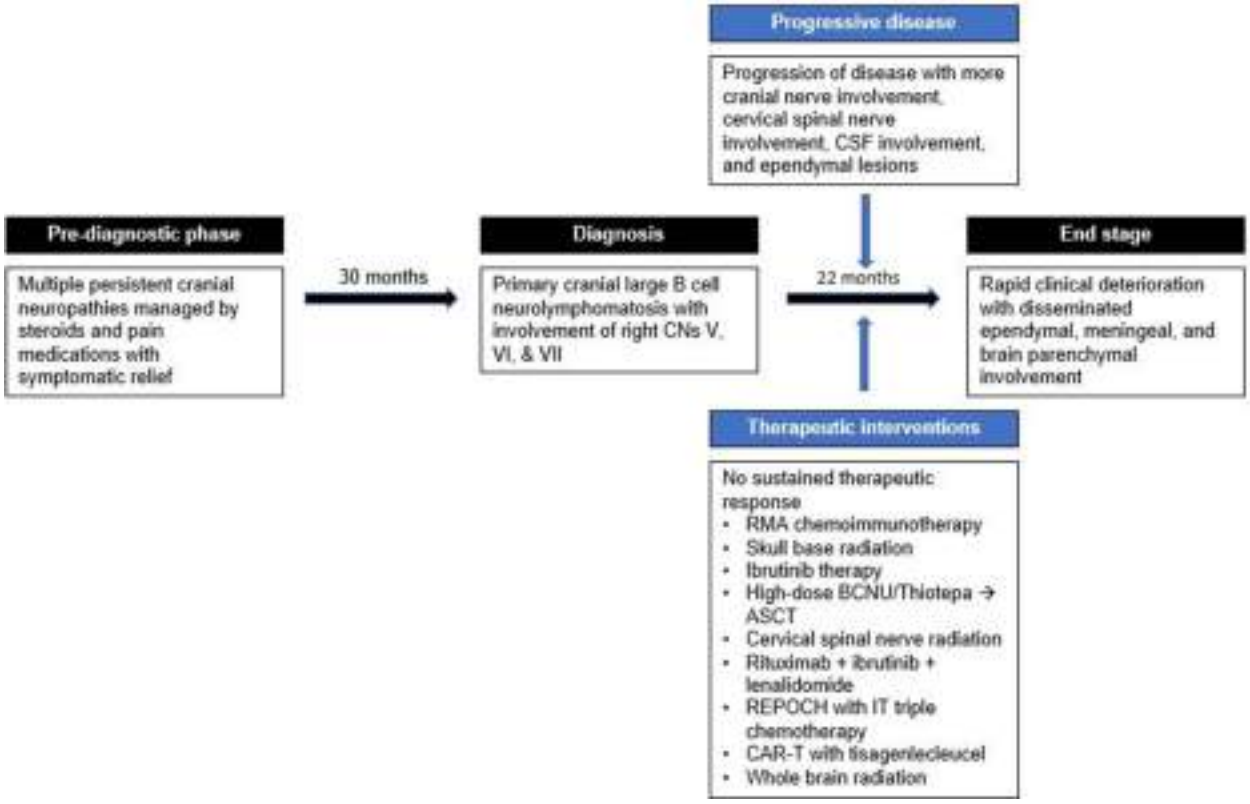

Figure 4 Diagram depicting the clinical timeline of our patient.

such, PCNL should be considered as a diagnostic possibility in patients with steroid-responsive but unexplained cranial neuropathies.

PCNL initially manifested in our patient with cranial neuropathies involving CN V, VI, VII, and VIII. He developed cervical spinal NL with CSF and brain involvement. Brain progression starts with ependymal tumors, followed by cerebral parenchymal tumors. A similar pattern of brain progression has been previously described.[1] In both cases, PCNL was initially observed in the brain as an ependymal tumor, followed by further dissemination in the brain parenchyma. This pattern of spread suggests that cerebral involvement occurs through the CSF pathway. Neither case showed evidence of PCNL via retrograde spread through the cranial nerves.

NL is difficult to diagnose in such cases. In the Massachusetts General Hospital (MGH) study covering the period 1972–2000, diagnosis was made by autopsy in 46% of the cases (33/72).[1] However, the IPCG study (1993–2008) showed a much lower incidence of diagnosis at autopsy at 8% (4/50), likely due to increased awareness and technology advancement.[2] In terms of diagnostic workup, MRI scans, PET scans, CSF analysis, and adequate biopsy of lesions on imaging are essential. PCNL appears as an enhancement along the tracts of cranial nerves. CSF cytology can be positive in approximately 40% of the cases.[1,2] PET can be used to rule out systemic lymphoma and peripheral nerve involvement. Collaboration with neurosurgical and radiology colleagues is essential to obtain adequate biopsies.

To the best of our knowledge, this is the first report of PCNL with genomic profiling. These findings include an activated B cell (non-germinal center phenotype) signature with MYD88 L265P, CD79b, and PIM1 mutations, which are similar to the typical genomic signature in PCNSL.[6] In terms of the mutation profile, this case can be classified as the MCD/C5 subtype.[7] Our patient had 22 mutations affecting PIM1, indicating highly active aberrant somatic hypermutation (Figure 5). PIM1 mutations have been associated with resistance to Bruton's tyrosine kinase inhibitors in ABC (non-germinal center phenotype)-DLBCL.[8] We identified four deleterious missense mutations

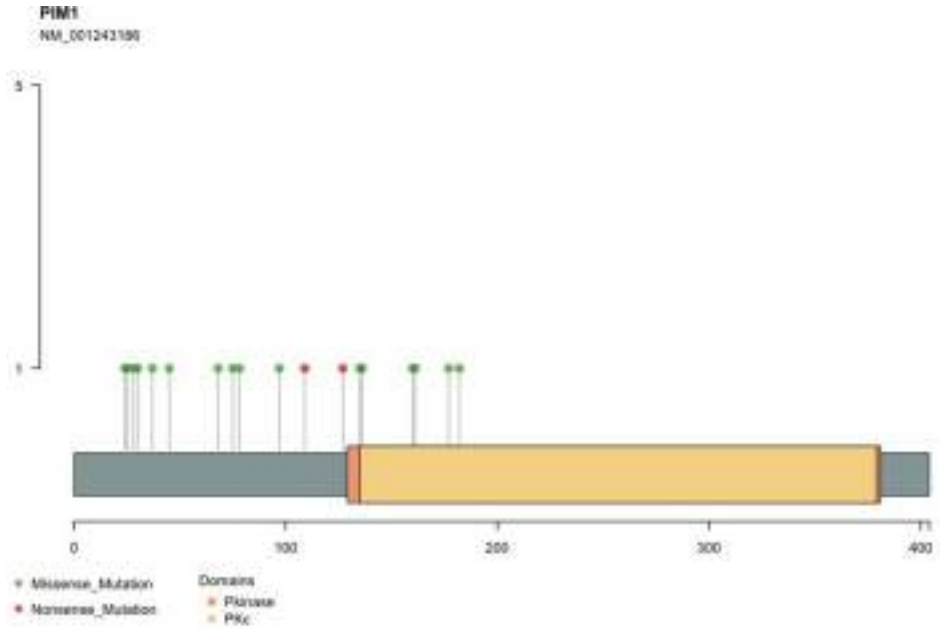

Figure 5 Lollipop plot of PIM1.

using SIFT and Polyphen prediction models (R136M, L182R, G45D, and G78R), two truncation mutations upstream of the functional kinase domain (W109* and Q127*), and four 5'UTR mutations.[9] 5'UTR mutations have been previously reported to result in overexpression of PIM1 and confer protection against autophagy.[10] Interestingly, the 5'UTR mutations were seen at lower allele frequencies than the non 5'UTR mutations (median 5'UTR Allele Frequency 7.11 (6.42–9.25) vs median non 5'UTR Allele Frequency 16.02 (7.86–36.48)). However, the significance of these findings remains unclear. Interestingly, our patient had two missense CXCR4 mutations that affected the carboxyl-terminus. This appears to be a unique feature of our case, as CXCR4 mutations have not been previously described in PCNSL.[11] The combination of MYD88, CD79, and CXCR4 mutations has been well-described in Waldenstrom macroglobulinemia (WM). Missense CXCR4 mutations in WM are associated with a more aggressive clinical course and resistance to ibrutinib.[12,13] Another unusual mutation found in our patient was IKZF3, which has not been previously described in PCNSL. Mutant IKZF3 in chronic lymphocytic leukemia has been associated with the hyperactive B-cell receptor signaling pathway and ibrutinib resistance.[14] Another novel finding was the TBL1XR1-ITPR1 fusion, which has not been described in the literature. TBL1XR1 mutations have been reported in 14–19% of PCNSL.[15,16] The profiling also revealed inversion 3, which has been previously described in myeloid leukemia and T-cell lymphoblastic leukemia/lymphoma.[17,18] The genomic profile in our case indicated highly upregulated signaling in B-cell receptors, Toll-like receptors, and NFKB signaling.

The patient did not respond to multiple lines of therapy in a sustained manner. Although there is no standardized approach for treating NL, it is reasonable to use the therapeutic approach adopted for PCNSL. HD-MTX based therapy appears to be associated with long-term survival in a subset of patients.[19–22] Survival has improved with the introduction of rituximab.[3] Radiation therapy was effective in terms of pain control, as observed in our patient. BTK inhibition was reasonable to consider for PCNSL, based on the MCD/C5 mutation profile. However, the ibrutinib therapy was ineffective. Thus, ASCT appears reasonable for eligible patients.[3] The blood-nerve barrier (BNB) appears to be structurally similar to the blood–brain barrier (BBB) and has been reported to be leakier.[23–25] BNB is also likely to be disrupted in the NL. In our case, R-EPOCH treatment had a disease-stabilizing effect, indicating its ability to permeate the BNB.

The findings of the literature review of the three largest NL studies are presented in Table 2. Cranial nerve involvement ranges 20–51% with CNS involvement 10–26%. 26–52% of the cases are primary NL. DLBCL

Table 2 Summary of the Three Largest NL Studies

	Mayo Study (2002–2018)	IPCG Study (1993–2008)	MGH Study (1972–2000)
Total number of cases (N)	40	50	72
Male	60%	60%	54%
Female	40%	40%	46%
Median age (Y)	60 Y	55.5 Y	63Y
B cell NHL	97%	82%	82%
DLBCL	68%	75.50%	NA
Cranial nerve involvement	20%	46%	51%
CNS involvement	10%	22%	26%
Primary NL	52%	26%	NA
Secondary NL	48%	74%	NA
HD-MTX therapy	62%	49%	12%
Rituximab	69%		
IT Chemotherapy	13%	49%	35%
Radiation therapy	13%	34%	72%
ASCT	40%	NA	NA
Median Overall Survival			
Whole Group	72.6 Months	10 Months (IY- 46%, 3Y- 24%)	NA
Primary NL	138 Months	20 Months	NA
Secondary NL	25.5 Months	8 Months	NA
Aggressive lymphoma	46.9 Months	NA	NA
Indolent lymphoma	Not reached	NA	NA

comprised 68–75.5% of the cases.[1–3] HD-MTX-based therapy was most frequently used in two recent studies.[1,3] The median overall survival for primary NL ranged from 20 (IPCG) to 138 months (Mayo). The Mayo series included patients with indolent B-cell lymphoma and peripheral nerve involvement. NL involvement in aggressive lymphoma was associated with a median OS of 46.9 months.[3]

We identified 12 cases of PCNL in the literature, with eight males and ages ranging from 33–74 (Table 3). All patients had B-cell lymphoma, with seven cases of DLBCL. The most common clinical manifestation was neurological facial symptoms seen in 8/12 patients with facial pain, numbness, or paralysis. The diagnosis was established by biopsy in 11/12 cases. CSF cytology was positive in 2/5 cases. HD-MTX-based therapy was used 7/12 cases. Complete response (CR) was reported in 8/12 patients. Long-term follow-up data was not available for all patients. Three patients died at 3, 14, and 18 months of follow-up. Two patients achieved CR at one-year follow up. One patient was in CR at 3-year follow up. One patient relapsed at eight months and was alive.

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Table 3 Summary of Reported Cases of PCNL

Sex/Age	Histologic Subtype	Affected CN	Symptoms	Biopsy Proven Diagnosis	CSF cytology	Treatment	Response to Treatment	Outcome	Reference
47 M	DLBCL	(R) CN V	Facial pain	Yes	Negative	R-MPV, WBRT	CR	Remission at 1 year follow up.	[26]
40 F	B-cell lymphoma	(L) CN V, VI	Facial numbness Facial pain Headaches Diplopia	Yes	Negative	R-CHOP, RT	NR	NR	[27]
55 M	DLBCL	(L) CN V	Facial pain	Yes	Positive	MTX, WBRT, whole spine RT, and local RT	CR	Died 14 months later with MRI concerning for CSF relapse	[28]
52 F	B-cell lymphoma	(L) CN V	Facial pain	Yes	NR	NR	NR	NR	[29]
50 M	B-cell lymphoma	(R) CN V, VI, VII,	Facial pain Facial numbness Facial palsy Diplopia	Yes	NR	MTX, ARA-C	CR	Remission at 1 year follow up	[19]
60 M	DLBCL	(L) CN V	Facial pain	Yes	NR	Gamma knife radiosurgery initially MTX, WBRT at relapse.	CR	Relapsed 8 months following gamma knife radiosurgery. Remained in remission at 30 month following treatment for relapsed disease.	[20]
55 F	B-cell lymphoma	(R) CN V	Diplopia	Yes	NR	NR	NR	NR	[30]
74 M	DLBCL	(L) CN IX and X	Dysphagia Hoarseness	Yes	NR	Local RT	CR	Relapsed 1 month after RT. Died 3 months following diagnosis.	[31]
65 M	DLBCL	(L) CN V	Facial pain	Yes	NR	MTX, local RT, WBRT initially R-CHOP at relapse	CR	Relapsed at 3 months. Died 18 months after initial diagnosis from pneumonia.	[32]

63 F	B-cell lymphoma	(L) CN V, VII	Diplopia Facial paralysis Facial pain Syncope Dysphagia Dysarthria	No	Positive	MTX-based chemotherapy	CR	Remission at 3 year follow up.	[21]
57 M	DLBCL	(L) CN V	Facial pain Hyperalgesia	Yes	Negative	MPV, Ferreri, aHSCT	CR	Remission at six month follow up.	[22]
33 M	DLBCL	Bilateral CN III and V	Hearing loss	Yes	NR	Chemotherapy (unspecified) and RT	NR	NR	[33]

Abbreviations: M, Male; F, Female; DLBCL, diffuse large B-cell lymphoma; L, left; R, right; CN, cranial nerve; NR, not reported; R-MPV, rituximab, methotrexate, procarbazine, and vincristine; WBRT, whole-brain radiation therapy; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiation therapy; MTX, methotrexate; ARA-C, cytarabine; MPV, methotrexate, procarbazine, and vincristine; Ferreri, methotrexate, cytarabine, and rituximab; aHSCT, autologous hematopoietic stem cell transplant; CR, complete response.

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Conclusion

Primary cranial NL is a rare subtype of PCNSL. Our patient shares a similar cell-of-origin signature (DLBCL-non-germinal center phenotype) and mutation profile with those of PCNSL (MYD88, CD79b, and PIM1 mutations). It is also associated with novel genomic alterations involving multiple mutations, such as PIM1, missense CXCR4 mutations, IKZF3 mutations, inversion 3, and TXL1XR1-ITPR1 fusion. The most common clinical symptoms are facial pain, numbness, and palsy, followed by diplopia. Therefore, PCNL should be considered in patients with unexplained steroid-responsive cranial neuropathies. Diagnostic evaluations should include MRIs, PET, CSF analysis, and biopsy. HD-MTX-based therapy has been frequently used in the management of NL in a subset of patients achieving long-term remission. Further research involving multiple institutions is needed to elucidate the biology and best therapeutic approach to PCNL.

Consent for Publication

The patient's next of kin has given written informed consent to publish the data, case details, and images. Institutional approval was not required to publish this case.

Disclosure

The authors report no conflicts of interest in this work.

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CASE REPORT

Management of Thrombosis in a Patient with Three Thrombophilic Disorders

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Abstract: Combined thrombophilia represents 7.8–8.3% of the patients with thrombophilia and confers a higher risk for thrombosis development and recurrence. Here, we present a 17-year-old boy carrier of three congenital thrombophilias, two severe (type I antithrombin deficiency and type I protein S deficiency) and one prothrombotic polymorphism (prothrombin G20210A), all in heterozygosis. He developed an extensive deep venous thrombosis in lower left limb, reaching proximal inferior vena cava and contralateral iliac vein, in the setting of prolonged rest. Endovascular therapy with local thrombolytic agent infusion followed by mechanical thrombectomy was performed, achieving a favorable clinical and radiological evolution. Antithrombin replacement to achieve levels between 80% and 120% with heparin administration was used during the endovascular procedure. The patient is currently asymptomatic and maintains indefinite anticoagulation with warfarin, keeping an appropriate anticoagulation range (international normalized range between 2.5 and 3.5).

Keywords: combined thrombophilia, deep venous thrombosis, inferior vena cava, endovascular therapy, anticoagulation

Introduction

Thrombophilia is defined as the disruption of the procoagulant and anticoagulant normal balance, leading to an abnormal coagulation tendency to clot formation.[1,2] Thrombophilia can be inherited or acquired.

Inherited prothrombotic disorders include common but mild prothrombotic polymorphisms: factor V Leiden and prothrombin G20210A, which require a trigger for thrombosis developments, such as prolonged immobilization, cancer, pregnancy or obesity.[2] On the other hand, rare and severe deficiencies of natural anticoagulants, including antithrombin (AT), protein S (PS) and protein C, are associated with a high risk of thrombosis. Other much rare severe congenital thrombophilias are plasminogen deficiency, hypo/dysfibrinogenemia, FIX Padua or prothrombin Yukuhashi.[1,3–5]

Among acquired thrombophilias, antiphospholipid syndrome is the most prevalent disorder.[6]

Thrombophilia can be identified in as many as 50% of the patients with venous thrombosis (VT) (35% of them with inherited thrombophilia).[1] Although all these thrombophilias play a role in thrombus development, the risk for initial thromboembolism varies, being hereditary AT deficiency the one with the highest thrombotic risk, although also with considerable clinical heterogeneity.[7]

The estimated prevalence of inherited AT deficiency in the general population lies between 0.02–0.2% and 1–5% in patients with thrombosis. The annual recurrence risk without long-term anticoagulation is 8.8% (95 CI 4.6–14.1) in AT-deficient VT patients.[8] The risk of thrombosis is related to the type of AT deficiency. Quantitative (type I) defects, when antigen and activity are similarly reduced, are usually associated with a high risk of thrombosis and recurrence. Different genetic defects cause type I deficiency: single nucleotide variants (SNVs) with non-sense, missense or splicing consequences, small insertions/deletions or structural variants. In contrast, qualitative (type II) defects, when antigen levels are normal but activity is reduced, are in general milder. Most genetic defects causing type II deficiencies are

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missense variants that depending on the functional consequences, define three subgroups: 1) type IIa or reactive site: mainly located in reactive center loop affecting the reactivity of AT; 2) type IIb or heparin-binding site, which impair heparin affinity; 3) type IIc or pleiotropic effect, if the mutation affects both the reactivity and heparin affinity.[9]

Mutations in *PROS1*, most of them SNVs, cause three types of hereditary PS deficiency: qualitative PS deficiency (type II), and two types of quantitative PS deficiencies: type I implies a reduction in both total and free levels, and type III when only free levels are reduced. In contrast to AT deficiency, the risk of thrombosis, which ranged 1- to 10-fold, did not change dependently on the type of PS deficiency.[1]

Thrombophilia is usually performed in patients with VT, particularly if they are under 50 years old, have recurrent episodes, thrombosis at unusual sites, or in asymptomatic subjects with a positive family hereditary thrombophilia.[10]

Combined thrombophilia is defined by the presence of more than one inherited defect in the same patient, constitutes 7.8–8.3% of the patients with thrombophilia, and confers a higher risk for thrombosis development and recurrence at an early age than single thrombophilia.[11,12]

Thus, patients with combined thrombophilia usually require long-term anticoagulation. Unfortunately, the rarity of severe combined thrombophilia had not allowed giving recommendations for the management of these patients. Another conflictive issue concerning patients with severe thrombophilia that affects carriers of combined thrombophilia is the anticoagulation to be used. Anticoagulation with vitamin K antagonists (VKA), heparin or direct oral anticoagulants (DOACs) is available. Although some published data suggest DOACs as an alternative to VKA in inherited thrombophilia,[1] the concerns for DOACs in patients with antiphospholipid syndrome, a severe acquired thrombophilia, strongly encourage further studies.[13]

Thrombolytic treatment, also known as fibrinolytic treatment (streptokinase, alteplase and urokinase are the drugs most used in clinical practice), dissolves pathologic thrombi to prevent ischemic damage and to improve blood flow.[14] Fibrinolytics can be administered systemically (intravenously) or locally (via catheter) and they have been mainly used in acute myocardial infarction and acute stroke, although they also have other less common indications including pulmonary embolism (PE) and deep venous thrombosis (DVT).[14–17]

Catheter-based endovascular techniques (either with local fibrinolytic agents or mechanical thrombectomy or the combination of both) improve clinical results in extensive DVT and preserves valvular vein competence, preventing or at least reducing post- thrombotic syndrome (PTS). The extent and location of the thrombus may alter the natural history of the DVT and should be considered when choosing the treatment. Anticoagulation prevents thrombus propagation. However, thrombus removal is achieved by the body´s own endogenous lytic capacity, which can be easily overcome by the large burden of an ilio-femoral DVT. The presence of massive proximal acute DVT in lower extremities accompanied by severe symptomatic leg swelling can be a feasible option for endovascular treatment and could be helpful in relieving PTS symptoms.[18,19] Enden et al included 189 patients with iliofemoral DVT, and the use of catheter-directed thrombolysis (CDT) was associated with a 26% reduction of PTS risk development.[20]

The ultrasound-accelerated CDT (USACDT) combines high frequency and low power ultrasound energy with thrombolytic therapy to achieve clot dissolution. Farrokhi et al in a meta-analysis of 18 studies included 597 participants and compared USACDT versus conventional CDT for DVT. They concluded that the success rate was significantly higher in USACDT. Although the mean infusion time and the rate of complications were lower in USACDT, statistical significance was not achieved.[21]

Additionally, bleeding risk is higher in patients treated with thrombolytic agents than in those treated with anticoagulants alone. Recently, major bleeding reported in the CaVenT (Catheter-Directed thrombolysis for Deep Vein Thrombosis) and the Acute Venous Thrombosis (Thrombus Removal with Adjunctive Catheter-Directed Thrombolysis (ATTRACT)) studies were 3% and 1.7%, respectively.[20,22] However, most procedure-related bleedings were minor and located at the access site.[23]

The identification of new cases with combined thrombophilia and the description of their clinical management will be useful to gain knowledge on this rare but severe thrombophilia. Here, we present a case with three prothrombotic defects who developed extended DVT in left lower limb that had a favorable clinical and radiological evolution with fibrinolysis, mechanical thrombectomy and anticoagulation.

Case Report

A 17-year-old male was admitted to the emergency department for assessment of pain and enlargement of the left lower extremity for 24 hours. The patient described prolonged rest 3 days before admission to our clinic due to lumbar pain. He had no cardiovascular risk factors and referred no previous personal thrombotic events. At the age of 3, he was diagnosed of hereditary AT deficiency type I (AT activity: 47%, AT antigen: 40%) (reference range 80–120%), mild free and total PS deficiency (45% both) (reference range 70–120%) and prothrombin variant G20210A in heterozygosis, due to the broad thrombotic family history (Figure 1). His father developed a spontaneous thrombosis in the right lower extremity at the age of 30, with PTS associated with venous ulcers. The father is currently on indefinite anticoagulation with VKA due to hereditary AT deficiency and maintains an appropriate international normalized ratio (INR) range (2.5–3.5). No thrombotic recurrences have been reported in 12 years of anticoagulation. His mother carries a type I PS deficiency and the PT G20210A polymorphism in heterozygosis. Neither the mother nor maternal grandfather, sister and maternal aunt carrying these two prothrombotic defects had previous thrombotic events, although the maternal aunt reported 3 miscarriages.

Molecular characterization of AT deficiency revealed a heterozygous point mutation in intron 5 of *SERPINC1* (NM_000488.4): c.1154–14G>A already described in Human Gene Mutation Database (HGMD) in other patients with type I deficiency (CS941423; rs542881762), which creates a cryptic splicing signal in the intron responsible for a variant with four in-frame additional residues that cause intracellular polymerization and traces of disulfide-linked dimers in plasma.[24,25]

Sequencing of *PROS1* revealed a relatively common heterozygous mutation (NM_000313.4): c.1501T>C (p. Ser501Pro), responsible for the PS Heerlen variant (CM951058) that slightly increases the risk of thrombosis.[26]

In the emergency department, edema in the left lower extremity as well as pain on mobilization and palpation were objectified. The blood test showed a high reactive C protein (9.27 mg/dL) (reference range <0.50 mg/dL), 19000 leukocytes (reference range 4000–11000) (86% neutrophils) and a D dimer of 84.23 µg/mL (reference range <0.50 µg/mL). The ultrasound revealed an increase in the diameter and echogenic content in the tibial, peroneal, popliteal, femoral, external iliac and common iliac veins, in relation to extensive DVT. Anticoagulation with low molecular weight

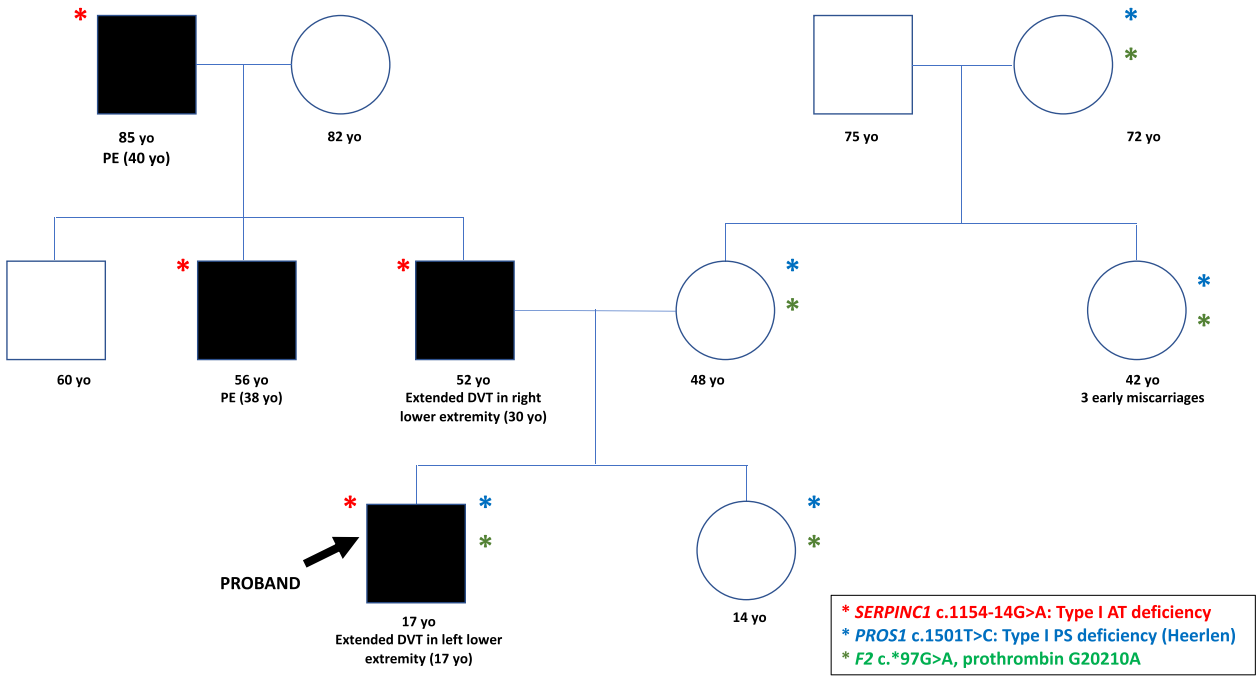

Figure 1 Pedigree of the studied family. Filled symbols represent thrombotic events. The age (yo: years old) of the thrombotic events is between brackets. The arrow points the proband. The prothrombotic genetic defects identified in each subject (all heterozygous) are also indicated.
Abbreviations: DVT, deep venous thrombosis; PE, pulmonary embolism.

heparin (LMWH, enoxaparin 1 mg/kg every 12 hours) was initiated. While the patient received heparin, AT replacement (50 IU/kg) to achieve an AT level between 80% and 120% was required to reach an optimal anticoagulation (target anti-Xa activity between 0.7–1.2 IU). For this purpose, anti-Xa activity and AT activity were measured. Abdominal computed tomography (CT) showed a DVT in the left lower limb, reaching the proximal inferior vena cava (IVC) and occupying 50% of the contralateral iliac vein at the confluence area, with moderate soft tissue edema. Also, the thoracic CT angiography findings were compatible with acute segmental PE (Figure 2).

Given the high probability of PTS in the setting of extensive DVT, percutaneous treatment was initially proposed. Just before the endovascular treatment, AT supplementation (50 UI/Kg) to reach an AT activity close to 100% was administered.

At the beginning of the procedure, an IVC filter was placed, and an ultrasound-guided puncture in the left posterior tibial vein was performed. A multi-side-hole infusion USACDT was placed within the thrombosed segment from the iliac to the distal popliteal (Figure 3).

To reduce the risk of bleeding, a low-dose fibrinolytic regime infusion rate of 0.6 mg per hour was used, and it was maintained for 24 hours with a total dose of 16 mg of rt-PA. Anticoagulation with unfractionated heparin was required to ensure an easier anticoagulation adjustment, depending on activated partial thromboplastin time (APTT) and fibrinogen levels.

In agreement with the local hospital protocol, during fibrinolytic infusion, fibrinogen should be checked every 4 hours while the patient receives rt-PA. If fibrinogen <200 mg/dl, the rt-PA is reduced to half the dose, while the rt-PA pump should be discontinued if fibrinogen <100 mg/dl.

The following day, the control phlebography revealed a significant improvement, with the remains of thrombus in the femoral, common femoral and popliteal veins. A pulse-pressure mechanical thrombectomy was then performed in the affected areas, achieving permeability and adequate flow without residual thrombosis. May–Thurner type venous

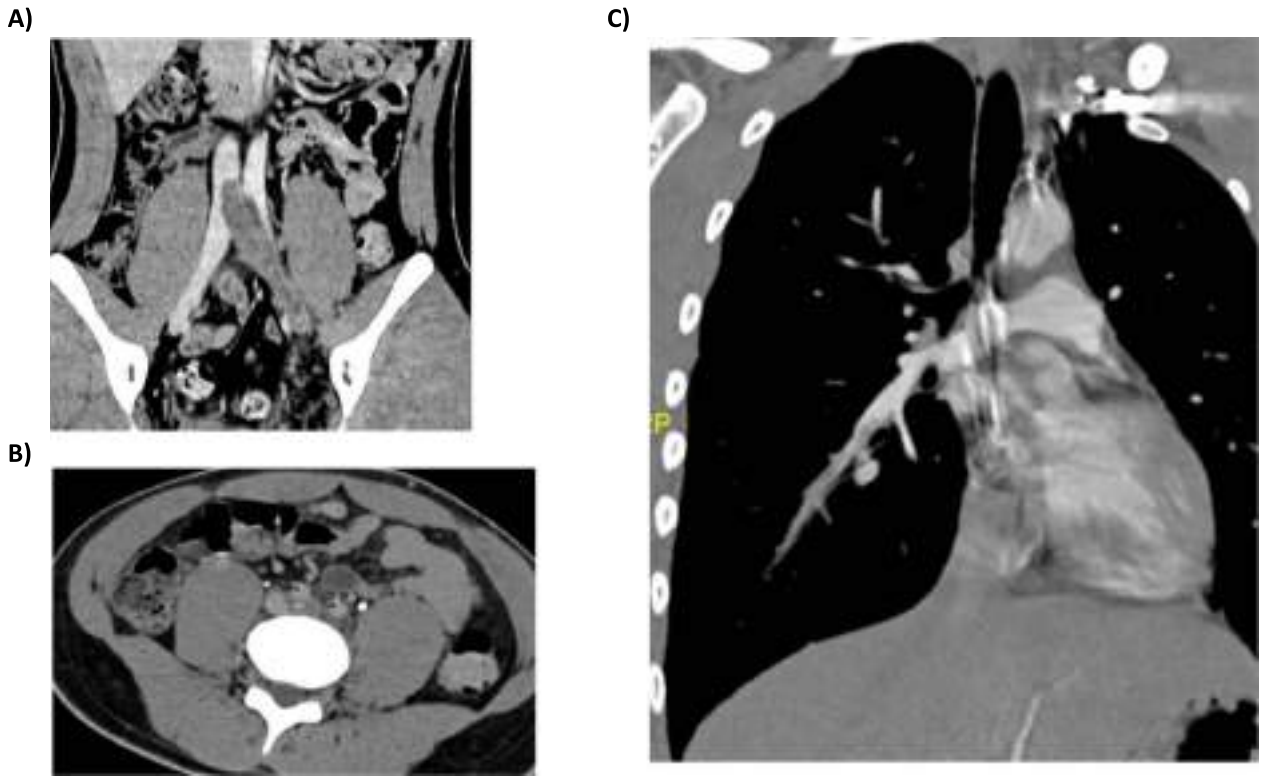

Figure 2 CT angiography findings at diagnosis. (**A**) Thrombosis in the left iliac vein that appears in the IVC; (**B**) Thrombosis in the left iliac vein without clear compression syndrome at the crossing behind the right iliac artery; (**C**) PE in branches of the right basal pyramid artery.
Abbreviations: CT, computer tomography; LIV, left iliac vein; RIA, right iliac artery; LIA, left iliac artery; IVC, inferior vena cava; PE, pulmonary embolism.

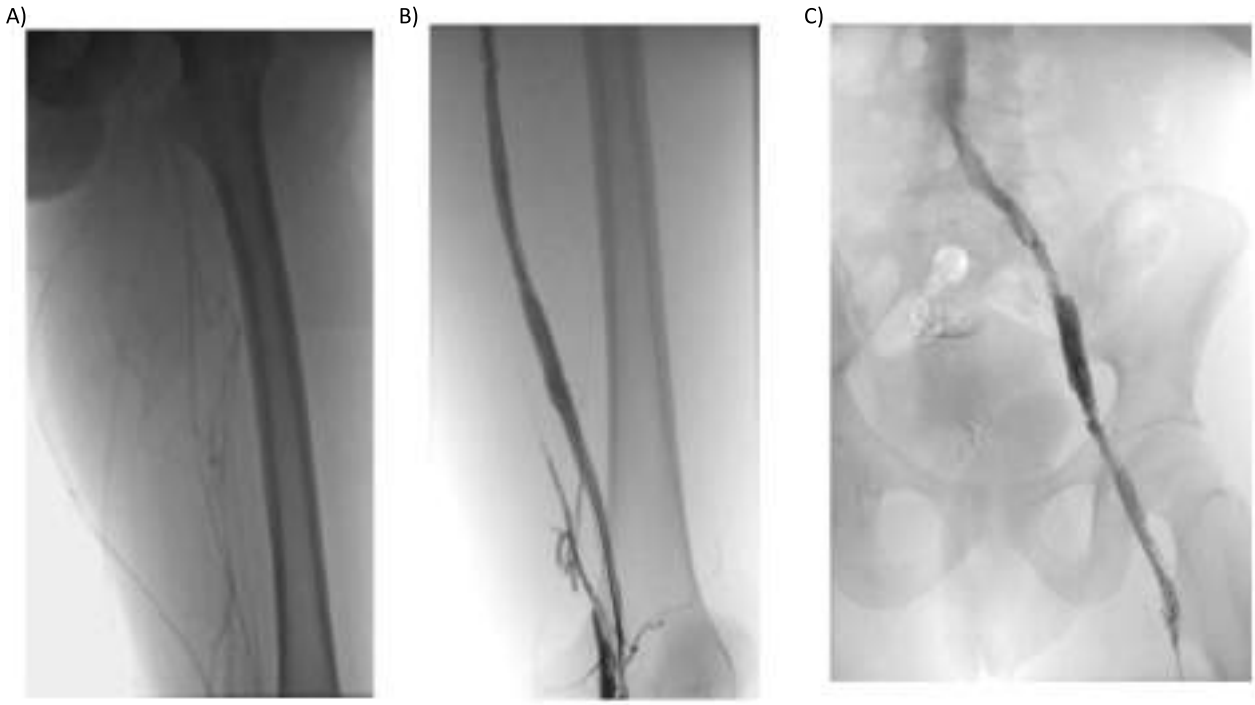

Figure 3 Phlebography from the posterior tibial vein. (**A**) Before rt-PA infusion: occupation of the deep tibial, popliteal and femoral venous territory, with little drainage through the superficial vein territory; B and (**C**) Following rt-PA infusion: permeability of the posterior tibial, popliteal, femoral, iliac and cava venous system.

compression was not confirmed in the final phlebography, so a stent was not required. Before finishing the procedure, the IVC filter was then removed.

The patient did not refer pain or bleeding and he initiated VKA (warfarin) together with bridge therapy (LMWH at therapeutic doses). The INR and anti-Xa activity were closely measured (every 12–24 hours) until two determinations in the therapeutic range were obtained. Considering the extensive thrombosis and the triple thrombophilia, we considered an optimal INR range between 2.5 and 3.5.

Nine months later, the angio-CT revealed total resolution of the PE (Figure 4). One year after the acute episode, no DVT in lower limbs was described in control ultrasounds. The patient is currently asymptomatic, and the INR remains in therapeutic range. Due to the high-risk multiple thrombophilia, the patient requires long-term anticoagulation. He referred adequate treatment adherence and no further adverse events have been reported.

Case Discussion and Review of the Literature

Up to 10% of the population is affected by one or more currently known inherited thrombophilia.[27] However, combined thrombophilic defects are pretty rare, representing 7.8–8.3% of the patients with thrombophilia.[12] Affected patients usually develop thrombosis at an early age and require long-term anticoagulation due to the high recurrence risk.[11,12]

The family shown here is an example of the clinical heterogeneity of thrombophilic factors and the consequence of their combination. The father, carrier of type I AT deficiency, according to the high risk of thrombosis of this deficiency had a thrombotic event at the age of 30 years. In contrast, the mother, his sister, his aunt, and his grandmother, despite carrying two prothrombotic defects (PS Heerlen and PT G20210A), had no thrombotic events, probably because these two defects are milder, and they did not have any cardiovascular risk factors. However, the proband, carrier of the three prothrombotic defects, had the most severe clinical phenotype according to the age of the first event (the patient was 17 years old).

Although the patient carries the highest thrombophilia risk (hereditary AT deficiency type I) in addition to 2 more mild to moderate inherited thrombophilia traits (PS deficiency and PT G20210A mutation in heterozygosis), he had not required previous thromboprophylaxis with LMWH, as he did not go through any prothrombotic settings like

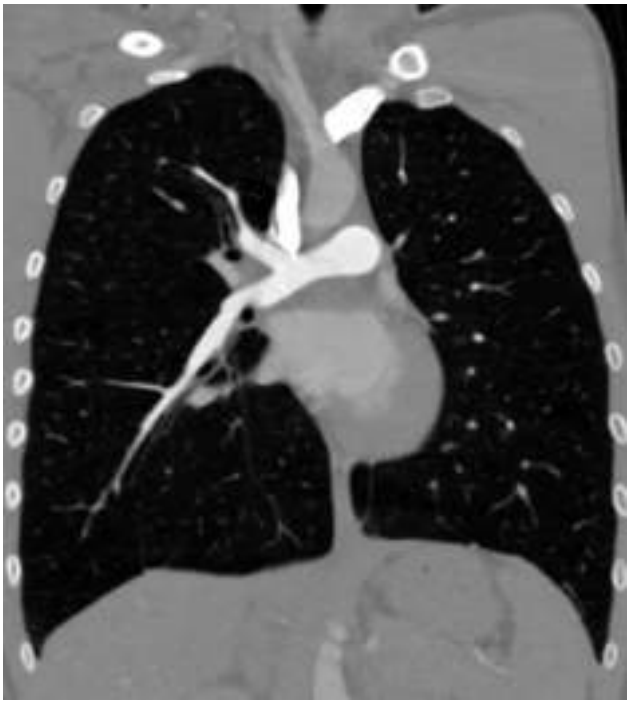

Figure 4 CT angiography findings 9 months following the initial diagnosis: complete resolution of the PE.
Abbreviations: CT, computed tomography; PE, pulmonary embolism.

immobilization or high-risk thrombotic surgery. But, in the setting of prolonged rest due to lumbar pain, he developed an extended DVT in the left leg, reaching the IVC.

The experience obtained with the management of the thrombotic event in the proband may also help the treatment of new cases with combined thrombophilia. Anticoagulation is the standard of care for thromboembolic disease. It helps reduce clot formation and prevent PE.[22] However, chronic complications derived from VT, especially PTS, may reach up to 40% of the affected individuals and imply a worsening in quality of life.[28] The rapid thrombus removal may improve deep venous flow and hence decreases PTS incidence.[23] Endovascular therapies, including local fibrinolysis, mechanical or combined strategies improve acute symptoms and reduce chronic sequelae, leading to higher rates of vein patency and preservation of valve function compared to anticoagulation alone.[18,19,23] Thrombolysis enhances immediate clot removal, and drugs such as urokinase and rt-PA are infused into a vein or locally using a catheter guided technique. CDT or pharmacomechanical CDT are likely to be safer and more effective than systemic thrombolytic therapy and could hold promise in preventing PTS. CDT is a promising option for massive and submassive PE with hemodynamic instability due to its rapid reversal of right ventricular dysfunction in people with acute PE compared to anticoagulation alone.[29] However, regarding DVT in lower extremities, the improvement in PTS symptoms is controversial and individualized treatment should be made.[18,19] When choosing endovascular therapies, several factors including age, severity and duration of symptoms, anatomical distribution of the thrombosis, response to systemic anticoagulation and bleeding risk should be considered.[17] Enden et al included 189 patients with iliofemoral DVT, and the use of CDT was associated with a 26% reduction of PTS risk development and 3% rate of major bleeding.[20] Liu et al included 38 patients with extensive DVT in lower extremities and reported a 90% complete lysis and no major bleedings. Competent femoral valves were observed in 86% of the patients. The PTS rate was 17% during a mean 20-month follow-up.[30]

There is scarce data regarding the use of DOACs in patients with combined thrombophilia,[31] In this setting, we considered AVK as the best anticoagulation option.

Treatment of iliofemoral DVT can be challenging. The use of anticoagulation alone can be insufficient for restoring venous competence. Symptomatic proximal DVT seems to be an optimal scenario for catheter-based endovascular techniques to clear the thrombus and prevent PTS development.

To sum up, patients with DVT in lower extremities should be carefully evaluated on a case-by-case basis. Those with acute symptomatic iliofemoral DVT, low bleeding risk and long-life expectancy are expected to be optimal candidates to combine anticoagulation and endovascular treatment.

Conclusions

- Combined thrombophilia is rare and increases thrombotic risk.
- Endovascular treatment can be a feasible option in proximal symptomatic DVT in lower extremities.

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.

Acknowledgments

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Disclosure

The authors declare no conflicts of interest in this work.

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REVIEW

Telomere Dynamics in Sickle Cell Anemia: Unraveling Molecular Aging and Disease Progression

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Abstract: Sickle Cell Anemia (SCA) is a hereditary blood disorder characterized by the presence of abnormal hemoglobin, leading to the formation of sickle-shaped red blood cells. While extensive research has unraveled many aspects of the genetic and molecular basis of SCA, the role of telomere dynamics in disease progression remains a relatively unexplored frontier. This review seeks to provide a comprehensive examination of telomere biology within the context of SCA, aiming to elucidate its potential impact on molecular aging and the progression of the disease. The impact of oxidative stress on telomere dynamics in SCA is explored, with a particular focus on how increased reactive oxygen species (ROS) may contribute to accelerated telomere shortening and genomic instability. Furthermore, the potential relationship between telomere dysfunction and cellular senescence in SCA is investigated, shedding light on how telomere dynamics may contribute to the premature aging of cells in this population. The review concludes by summarizing key findings and proposing potential therapeutic strategies targeting telomere dynamics to mitigate disease progression in SCA. It also identifies gaps in current understanding and suggests avenues for future research, emphasizing the importance of further investigating telomere biology to advance our understanding of molecular aging and disease progression in Sickle Cell Anemia. This comprehensive exploration of telomere dynamics in SCA offers insights into potential mechanisms of molecular aging and disease progression, paving the way for targeted therapeutic interventions and improved disease management.

Keywords: sickle cell anemia, telomere, molecular aging, hemoglobinopathy, telomere shortening, oxidative stress

Introduction

Sickle Cell Anemia (SCA) stands as a paradigmatic hematological disorder, characterized by the abnormal production of hemoglobin and the consequential formation of sickle-shaped erythrocytes.[1–3] While advancements in genetic and molecular research have significantly enhanced our understanding of SCA, certain facets of its pathophysiology, particularly the role of telomere dynamics, remain relatively unexplored. Telomeres, protective nucleoprotein structures at the ends of chromosomes, play a crucial role in preserving genomic integrity and cellular function.[4] Given their significance in normal cellular processes, disruptions in telomere dynamics may have far-reaching consequences. This review endeavors to provide a comprehensive exploration of telomere biology within the context of SCA, aiming to unravel its potential implications for molecular aging and disease progression. Understanding the molecular intricacies of SCA, coupled with insights into telomere dynamics, holds the promise of shedding light on novel mechanisms influencing the course of the disease. SCA arises from a point mutation in the β-globin gene, leading to the synthesis of abnormal hemoglobin (HbS). The resultant sickle-shaped erythrocytes contribute to vaso-occlusive events, hemolysis, and a myriad of clinical complications. While much attention has been given to the genetic determinants and molecular events underpinning SCA, the impact of telomere dynamics on disease progression remains an area warranting exploration.[5,6] Telomere dysfunction has been implicated in various hematological and aging-related disorders. Understanding its role in SCA could provide novel insights into the molecular mechanisms driving the disease.[7] This review is prompted by the need to synthesize existing knowledge on telomere dynamics in SCA, elucidating their potential contribution to molecular aging and disease progression.

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Sickle Cell Anemia (SCA) is a complex disorder characterized by the presence of abnormal hemoglobin S, which leads to the sickling of red blood cells. This sickling process triggers a cascade of pathophysiological mechanisms including chronic hemolysis, vaso-occlusive events, and widespread inflammation. These processes impose considerable stress on various cell types, particularly hematopoietic stem cells (HSCs), which are responsible for the continuous replenishment of blood cells. The high turnover rate of these cells, combined with persistent oxidative stress and inflammatory signals, results in accelerated telomere shortening in individuals with SCA. Telomeres, which protect chromosome ends from deterioration, naturally shorten with each cell division. However, in SCA, this shortening is exacerbated, leading to premature cellular senescence or apoptosis, thereby contributing to the overall disease burden and progression. The advent of gene therapy for SCA holds significant promise in addressing telomere shortening-associated aging. By correcting the underlying genetic defect, gene therapy can potentially reduce the chronic hemolysis and inflammation that drive excessive cell turnover and oxidative stress. Consequently, this reduction could alleviate the accelerated telomere attrition observed in SCA patients. By stabilizing telomere length, gene therapy may not only improve hematopoietic stem cell function and longevity but also mitigate the premature aging and comorbidities associated with SCA. This therapeutic approach represents a transformative shift in managing SCA, offering hope for improved quality of life and prolonged health span for patients suffering from this debilitating condition.[2,3]

Recent advancements in gene therapy have ushered in a new era of potential treatments for Sickle Cell Anemia (SCA), offering hope for addressing the genetic root of this debilitating disease. Traditional therapies have primarily focused on managing symptoms and complications, but gene therapy aims to correct the underlying genetic defect, thereby altering the disease trajectory fundamentally. One of the most promising approaches involves using CRISPR-Cas9 technology to edit the HBB gene, thereby correcting the mutation responsible for the production of sickle hemoglobin. By targeting the source of hemoglobin abnormalities, gene therapy could significantly reduce hemolysis, inflammation, and oxidative stress—all of which are major contributors to telomere shortening in SCA patients. The potential impact of successful gene therapy on telomere dynamics in SCA patients could be profound. As gene therapy reduces the burden of sickled red blood cells, it is likely to decrease the chronic inflammatory state and oxidative damage that drive telomere attrition. Consequently, patients might experience a slowdown in the accelerated telomere shortening associated with SCA, potentially mitigating premature cellular aging and reducing the risk of age-related comorbidities. Additionally, by preserving telomere length, gene therapy could enhance the longevity and functionality of hematopoietic stem cells, thereby improving the overall hematopoietic system's resilience and reducing the frequency of complications such as vaso-occlusive crises. This could not only extend the lifespan of SCA patients but also significantly enhance their quality of life.[4–7]

Objectives

1. To provide a comprehensive overview of telomere structure, function, and dynamics.
2. To explore existing literature on normal telomere dynamics in healthy individuals.
3. To investigate and summarize evidence of altered telomere dynamics specific to SCA.
4. To assess the potential impact of telomere dysfunction on cellular senescence and disease progression in SCA.
5. To discuss therapeutic implications and identify potential avenues for future research.

A thorough understanding of telomere dynamics in SCA may offer new perspectives on the molecular underpinnings of the disease, paving the way for targeted therapeutic interventions and improved patient management. By bridging the knowledge gap between telomere biology and SCA, this review contributes to a broader comprehension of molecular aging and disease progression in this hematological disorder.

Telomere Structure and Function

Telomeres, the protective caps located at the ends of linear chromosomes, play a pivotal role in maintaining genomic stability and cellular function.[8] Composed of repetitive DNA sequences and associated proteins, telomeres safeguard chromosomal integrity, preventing degradation and end-to-end fusions. Telomeres consist of tandem repeats of a specific DNA sequence, typically TTAGGG in vertebrates.[9] These repeats form a protective overhang, often termed the G-rich

single-stranded 3' overhang, essential for telomere function. The unique composition of telomeric DNA distinguishes it from the coding regions of the genome. The shelterin complex, a group of six telomere-specific proteins (TRF1, TRF2, POT1, TIN2, TPP1, and RAP1), binds to telomeric DNA and orchestrates various functions.[10] TRF1 and TRF2 bind to double-stranded telomeric DNA, while POT1 binds to the single-stranded overhang. The complex collectively ensures telomere protection, regulation, and proper telomere length maintenance.

Telomerase is an enzymatic complex crucial for telomere maintenance.[11] Comprising a catalytic subunit (TERT) and an RNA template (TERC), telomerase extends telomeric DNA, compensating for the gradual loss during cellular replication.[12] While active in germ cells and certain stem cells, most somatic cells exhibit limited telomerase activity, contributing to telomere shortening with each cell division.

Telomeres act as a biological clock, determining the number of cell divisions a somatic cell can undergo. The gradual shortening of telomeres with each division culminates in cellular senescence or apoptosis. By preventing chromosomal end-to-end fusions, telomeres maintain genomic stability and integrity. Dysfunctional telomeres can lead to chromosomal aberrations and genomic instability.[8] Critically short telomeres activate DNA damage responses, leading to cellular senescence or apoptosis.[13] This serves as a protective mechanism against the propagation of damaged cells. Telomere shortening is an inherent consequence of cellular replication, occurring in the absence of sufficient telomerase activity.[14] Factors influencing telomere shortening include oxidative stress, inflammation, and environmental exposures. Telomere attrition contributes to the aging process and is implicated in age-related diseases. Dysfunctional telomeres are associated with various diseases, including certain cancers and hematological disorders.[15] Telomere dysfunction can promote genomic instability, contributing to the initiation and progression of diseases. Understanding telomere dynamics holds therapeutic potential. Strategies targeting telomerase activation or modulation, known as telomerase-based therapies, are under investigation for age-related diseases and conditions associated with telomere dysfunction.

Telomere Dynamics in Healthy Individuals

Telomere dynamics play a crucial role in maintaining cellular health and functionality. In healthy individuals, telomere length varies among cell types, tissues, and individuals.[16] While telomeres are generally shorter in somatic cells compared to germ cells, a dynamic equilibrium exists, ensuring cellular homeostasis. Genetic and environmental factors contribute to inter-individual differences in telomere length. Telomerase, the enzyme responsible for telomere elongation, is predominantly active during embryonic development, in stem cells, and in certain specialized cells. In healthy somatic cells, telomerase activity is typically limited, leading to gradual telomere shortening with each cell division.[17] The Hayflick limit, proposed by Leonard Hayflick, describes the phenomenon of cellular replicative senescence, wherein somatic cells reach a maximum number of divisions due to telomere shortening.[18] This process acts as a natural biological clock, regulating the lifespan of cells. Aging is associated with cumulative telomere shortening, reflecting the historical cellular divisions experienced by an individual's cells.[19] The gradual loss of telomeric DNA contributes to the aging process, impacting tissue functionality and overall health. Various lifestyle factors, including stress, physical activity, and diet, can influence telomere dynamics.[20] Chronic stress and unhealthy lifestyle choices may accelerate telomere shortening, while positive lifestyle modifications and stress management strategies have been associated with telomere preservation.

Stem cells, characterized by the ability to self-renew and differentiate into various cell types, possess unique telomere maintenance mechanisms.[21] The balance between telomere shortening and preservation is crucial for the sustained regenerative capacity of stem cells. Immune cells, especially lymphocytes, undergo dynamic changes in telomere length throughout an individual's life.[22] Telomere shortening in immune cells is associated with decreased immune function and increased susceptibility to infections in the elderly. Studies suggest gender differences in telomere length dynamics, with women generally exhibiting longer telomeres than men.[23–25] Hormonal influences and X-chromosome inactivation may contribute to these variations. Despite the inevitable telomere shortening associated with aging, healthy individuals maintain telomere homeostasis through a delicate balance between telomere attrition, telomerase activity, and cellular turnover.[26]

Telomere Shortening in Sickle Cell Anemia

Sickle Cell Anemia (SCA) is a hereditary hemoglobinopathy characterized by abnormal hemoglobin production, leading to the formation of sickle-shaped erythrocytes. While much attention has been devoted to the genetic aspects of SCA, the impact of telomere dynamics on disease progression remains a less-explored aspect.[27–29] Emerging evidence suggests that individuals with SCA may experience accelerated telomere shortening compared to healthy counterparts.[30] Factors contributing to this phenomenon include chronic hemolysis, increased oxidative stress, and the heightened inflammatory state characteristic of SCA. Hemolysis, a hallmark of SCA, results in the premature destruction of erythrocytes. The chronic turnover of red blood cells, compounded by the unique challenges presented by sickle-shaped cells, may contribute to accelerated telomere attrition in individuals with SCA.[31–33] The oxidative stress inherent in SCA, driven by the presence of abnormal hemoglobin and inflammatory processes, poses a potential link to telomere shortening.[34] Increased reactive oxygen species (ROS) generation may contribute to DNA damage, affecting telomeric regions. The chronic inflammatory state in SCA, triggered by recurrent vaso-occlusive events and endothelial dysfunction, could contribute to telomere dysfunction. Inflammatory mediators may influence telomerase activity and exacerbate telomere shortening.[35] The unique bone marrow environment in individuals with SCA, characterized by increased erythropoiesis and altered hematopoietic niches, may impact telomere dynamics.[36] The continuous demand for new erythrocytes may contribute to accelerated telomere attrition in hematopoietic stem cells.

Telomerase in Sickle Cell Anemia

Telomerase, a key enzyme responsible for maintaining telomere length, plays a critical role in cellular homeostasis.[37] In the context of Sickle Cell Anemia (SCA), understanding the dynamics of telomerase activity becomes essential due to the unique challenges posed by the disease. Most somatic cells exhibit limited telomerase activity, leading to gradual telomere shortening with each cell division.[38] Factors such as chronic hemolysis, oxidative stress, and inflammatory processes may influence the regulation of telomerase in SCA patients.[39] Given the elevated oxidative stress in SCA, this section delves into the potential influence of reactive oxygen species (ROS) on telomerase regulation. Oxidative stress is known to impact telomere dynamics, and understanding this interplay in SCA is essential. Inflammatory mediators may modulate the regulation of telomerase, influencing its function in SCA.

Oxidative Stress and Genomic Instability

Oxidative stress and genomic instability are interconnected processes that play crucial roles in various physiological and pathological conditions.[40] Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them through antioxidants.[41] Reactive oxygen species are generated during normal cellular metabolism, and their levels can increase due to factors such as environmental pollutants, radiation, inflammation, and certain drugs. Excessive ROS can damage cellular components, including lipids, proteins, and DNA, leading to disruptions in normal cell function and contributing to the development of various diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases.[42] Genomic instability refers to an increased tendency for alterations in the DNA sequence, such as mutations, deletions, and chromosomal rearrangements.[43] Genomic instability can result from various factors, including errors during DNA replication, exposure to mutagenic agents (such as certain chemicals or radiation), and defects in DNA repair mechanisms. Genomic instability is a hallmark of cancer, as it can lead to the accumulation of genetic mutations that drive the uncontrolled growth of cells. ROS can directly damage DNA by causing modifications to its structure.[44] For example, they can induce base modifications and strand breaks. Oxidative stress can also interfere with the normal functioning of DNA repair mechanisms. If the repair processes are compromised, the likelihood of genomic instability increases. Genomic instability can further contribute to oxidative stress. Mutated or damaged cells may produce more ROS, creating a cycle of damage and instability. The link between oxidative stress and genomic instability is particularly relevant in cancer.[45] Genomic instability, often driven by oxidative damage, can contribute to the initiation and progression of cancer.[46] Both oxidative stress and genomic instability are associated with aging and age-related diseases, including neurodegenerative disorders.

Association with SCA Manifestations/Pathophysiology and Telomere Shortening Specifying the Mechanisms

The association between Sickle Cell Anemia (SCA) manifestations and telomere shortening is multifaceted, with various mechanisms intertwining to exacerbate the disease pathology. SCA is marked by chronic hemolysis and vaso-occlusive episodes, leading to continuous cycles of tissue ischemia and reperfusion. These pathological processes contribute significantly to oxidative stress, inflammation, and increased cellular turnover, all of which play pivotal roles in telomere shortening. Oxidative stress is a critical factor in telomere shortening within SCA. Due to recurrent hemolysis, free heme and iron are released into the circulation, catalyzing the formation of reactive oxygen species (ROS). ROS can cause direct damage to DNA, including telomeric regions, which are particularly susceptible to oxidative damage due to their high guanine content. This oxidative damage accelerates the shortening of telomeres, leading to premature cellular senescence and apoptosis. Chronic inflammation is another major contributor to telomere attrition in SCA. Persistent inflammatory responses are driven by ongoing tissue damage and the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1, IL-6). These cytokines promote the activation and proliferation of immune cells, particularly leukocytes. The increased turnover and proliferation rates of these cells lead to heightened telomere shortening. Additionally, inflammatory mediators can induce oxidative stress, further contributing to telomere erosion.[41–43]

In SCA, there is a marked increase in cellular turnover, particularly within the hematopoietic system. The chronic destruction of sickle-shaped erythrocytes necessitates constant replenishment from hematopoietic stem cells (HSCs). This relentless demand for new blood cells imposes significant replicative stress on HSCs, resulting in accelerated telomere shortening. Over time, this can lead to HSC exhaustion, diminishing the bone marrow's capacity to produce adequate blood cells and contributing to the anemia and other hematological abnormalities seen in SCA patients. Endothelial cells lining the blood vessels are also adversely affected by telomere shortening in SCA. The repetitive cycles of ischemia-reperfusion injury led to endothelial activation and dysfunction. Endothelial cells with critically short telomeres may become senescent or apoptotic, compromising vascular integrity and function. This dysfunction exacerbates the frequency and severity of vaso-occlusive crises, a hallmark of SCA. Telomere shortening has direct implications for various clinical manifestations of SCA. Shortened telomeres in HSCs and progenitor cells can lead to ineffective hematopoiesis, contributing to severe anemia and increased transfusion requirements. Furthermore, the premature aging of immune cells due to telomere attrition can impair immune responses, increasing susceptibility to infections. Vascular complications, such as pulmonary hypertension and chronic kidney disease, are also linked to telomere shortening through mechanisms involving endothelial dysfunction and vascular aging.[44,45]

Telomeres and Senescence in Sickle Cell Anemia

Telomeres and senescence are important cellular processes that can be relevant to the understanding of sickle cell anemia, a genetic disorder characterized by abnormal hemoglobin in red blood cells. Telomeres gradually shorten with each cell division, and when they become critically short, cells may enter a state of replicative senescence or undergo apoptosis (cell death).[47] In individuals with sickle cell anemia, red blood cells are prone to premature destruction (hemolysis), leading to a higher turnover of red blood cells. This increased turnover could potentially impact telomere length over time. Cellular senescence is a state in which cells cease to divide and undergo functional changes.[48] It can be triggered by various factors, including DNA damage, telomere shortening, and stress. The chronic nature of sickle cell anemia, characterized by recurrent episodes of vaso-occlusive crises and inflammation, may contribute to cellular stress and damage, potentially triggering senescence in various cell types.

The increased turnover of red blood cells in sickle cell anemia may contribute to telomere shortening over time, potentially affecting the lifespan of these cells.[49] The inflammatory nature of sickle cell anemia, coupled with oxidative stress, could contribute to cellular senescence in different tissues. Senescent cells may release pro-inflammatory signals, creating a feedback loop that exacerbates inflammation. Telomere shortening and cellular senescence may play roles in the overall pathophysiology of sickle cell anemia.[50] Premature aging of cells, particularly those involved in the immune response or tissue repair, could influence the severity and complications of the disease. Understanding the interplay

between telomeres, senescence, and sickle cell anemia may identify potential therapeutic targets. Strategies aimed at mitigating oxidative stress, inflammation, and improving cellular repair mechanisms could be explored.

Therapeutic Implications and Future Directions

Therapeutic implications for conditions involving oxidative stress, genomic instability, telomeres, senescence, and diseases like sickle cell anemia are broad and multifaceted.[51] Developing and utilizing antioxidant therapies to mitigate oxidative stress, which may help in conditions where ROS-induced damage is a significant factor.[52] Augmenting DNA repair mechanisms to address genomic instability.[53] Exploring interventions to maintain or lengthen telomeres to slow down cellular aging. Developing drugs to modulate senescence, either by promoting the clearance of senescent cells (senolysis) or altering the senescence-associated secretory phenotype (SASP).[54] Utilizing stem cell therapies to replace damaged or senescent cells and promote tissue regeneration.[55] Tailoring treatments based on the individual's genetic makeup, considering variations in genes related to oxidative stress response, DNA repair, telomere maintenance, and other relevant pathways.[56] Modulating the immune system to better respond to cellular damage and improve the clearance of damaged or senescent cells.[57] Promoting healthy lifestyles that include a balanced diet, regular exercise, and stress management to reduce the overall burden of oxidative stress and promote overall well-being. Continued development of advanced technologies such as gene editing (eg, CRISPR-Cas9) for precise modification of genes associated with these processes.[58,59]

Up-to-Date Knowledge and Approaches to Gene-Editing for Sickle Cell Anemia Treatment

Gene editing has emerged as a promising therapeutic approach for Sickle Cell Anemia (SCA), a genetic disorder caused by a mutation in the HBB gene encoding the beta-globin subunit of hemoglobin. This mutation leads to the production of abnormal hemoglobin S (HbS), causing red blood cells to become sickle-shaped and prone to hemolysis, leading to various complications. Recent advancements in gene-editing technologies, particularly CRISPR-Cas9, have opened new avenues for correcting the genetic defect at its source, offering potential cures for SCA.[58]

CRISPR-Cas9 Technology

CRISPR-Cas9 is a revolutionary gene-editing tool that allows for precise modifications of specific DNA sequences. In the context of SCA, CRISPR-Cas9 can be used to target and correct the HBB gene mutation or to induce the expression of fetal hemoglobin (HbF), which can ameliorate the disease symptoms.

1. **Direct Correction of HBB Mutation**: The CRISPR-Cas9 system can be designed to specifically target the mutant HBB gene and correct the single nucleotide mutation responsible for SCA. This approach involves the use of guide RNA (gRNA) to direct the Cas9 nuclease to the precise location of the mutation, where it introduces a double-strand break. The cell's repair mechanisms then use a supplied DNA template to repair the break, correcting the mutation and restoring normal beta-globin production.
2. **Induction of Fetal Hemoglobin (HbF)**: Another strategy leverages the natural protective effects of HbF, which inhibits HbS polymerization. CRISPR-Cas9 can be used to disrupt repressors of HbF expression, such as BCL11A or the HBG promoter regions. By knocking out these repressors, HbF levels can be increased, reducing the clinical severity of SCA.

Advances and Clinical Trials

Several clinical trials and preclinical studies have demonstrated the potential of CRISPR-Cas9-mediated gene editing for treating SCA. Notable examples include:

1. **CTX001 (CRISPR Therapeutics and Vertex Pharmaceuticals)**: CTX001 is an investigational therapy that uses CRISPR-Cas9 to edit the BCL11A gene in patients' hematopoietic stem cells (HSCs). By knocking out BCL11A,

the expression of HbF is reactivated. Early clinical trial results have shown promising outcomes, with treated patients exhibiting increased HbF levels and reduced SCA symptoms.

2. **Other Clinical Trials**: Several other clinical trials are underway, exploring various strategies for gene editing in SCA. These include approaches to directly correct the HBB mutation or enhance HbF production through different targets and delivery methods.

While gene editing offers exciting prospects, it also raises important safety and ethical considerations. Off-target effects, where unintended genetic modifications occur, are a primary concern. Ensuring the specificity and accuracy of gene-editing tools is crucial to minimize potential risks. Additionally, long-term monitoring of patients is necessary to assess the durability and safety of the edited cells. Ethical considerations include ensuring equitable access to these advanced therapies and addressing potential socio-economic disparities. Furthermore, informed consent and the ethical implications of germline editing, though not currently a focus for SCA, require careful consideration. The future of gene editing for SCA is promising, with ongoing research aimed at improving the efficiency, safety, and accessibility of these therapies. Innovations such as base editing and prime editing offer even more precise genetic modifications with potentially fewer off-target effects. Additionally, advancements in delivery methods, such as nanoparticles and viral vectors, are being explored to enhance the efficacy of gene-editing therapies. Combining gene editing with other therapeutic approaches, such as gene therapy and small molecule drugs, may also enhance treatment outcomes. Personalized medicine, where gene-editing strategies are tailored to individual patients' genetic profiles, represents another exciting frontier.[58,59]

Fresh Perspectives and Significant New Contributions to Gene-Editing for Sickle Cell Anemia

Recent advancements in gene-editing technologies have revolutionized the landscape of therapeutic options for Sickle Cell Anemia (SCA). While CRISPR-Cas9 has been the centerpiece of these innovations, newer approaches and insights are continually emerging, pushing the boundaries of what is possible in treating this debilitating genetic disorder. Here, we explore fresh perspectives and significant contributions that are shaping the future of gene-editing therapies for SCA.[2]

Base Editing: Precision Without Double-Strand Breaks

One of the most significant new contributions to gene-editing technology is the development of base editing. Unlike CRISPR-Cas9, which relies on creating double-strand breaks in DNA to induce repair mechanisms, base editors directly convert one nucleotide to another without making double-strand breaks. This method greatly reduces the risk of off-target effects and unwanted mutations.

1. **Adenine Base Editors (ABEs)**: ABEs convert adenine-thymine (A-T) base pairs to guanine-cytosine (G-C) base pairs. In the context of SCA, ABEs can precisely correct the single nucleotide mutation from adenine to thymine in the HBB gene that causes the production of HbS. This approach offers a safer and potentially more efficient method for correcting the genetic defect in SCA patients.
2. **Cytosine Base Editors (CBEs)**: Similarly, CBEs convert cytosine-guanine (C-G) base pairs to thymine-adenine (T-A) base pairs. While ABEs are directly relevant for SCA mutation correction, CBEs can be instrumental in other genetic modifications needed for SCA therapy, such as silencing repressor genes of fetal hemoglobin.[58]

Prime Editing: Versatility and Precision

Prime editing represents another groundbreaking advancement in the gene-editing field. This technology combines the specificity of CRISPR-Cas9 with a reverse transcriptase to directly write new genetic information into a target site, enabling a wide range of genetic alterations, including all possible base-to-base conversions, small insertions, and deletions.[59]

1. **Application in SCA**: Prime editing can be employed to correct the SCA mutation by precisely converting the abnormal thymine to adenine in the HBB gene. Its versatility allows for more complex genetic edits, which can be beneficial in addressing the heterogeneity of genetic mutations in SCA and potentially correcting multiple genetic defects in a single therapeutic intervention.

Enhancing Delivery Methods: Non-Viral Vectors and Nanoparticles

The delivery of gene-editing tools into patient cells, particularly hematopoietic stem cells (HSCs), is a critical challenge. Recent innovations in delivery methods are poised to significantly improve the efficiency and safety of gene-editing therapies.

1. **Non-Viral Vectors**: Advances in non-viral delivery systems, such as electroporation and lipid nanoparticles, are reducing the reliance on viral vectors, which can have immunogenicity and integration-related risks. These non-viral methods can offer safer and more controllable delivery of gene-editing components into HSCs.
2. **Nanoparticle Delivery**: Nanoparticles designed to encapsulate and protect CRISPR-Cas9 components are being optimized for efficient delivery into target cells. These nanoparticles can improve the precision of gene-editing tools, enhance cellular uptake, and minimize off-target effects by ensuring that the gene-editing components are delivered directly to the desired cellular compartments.

Synthetic Biology and Gene Circuits

Synthetic biology approaches, including the design of gene circuits, are contributing novel ways to regulate gene expression and enhance the safety of gene-editing therapies.

1. **Programmable Gene Circuits**: These circuits can be designed to regulate the expression of therapeutic genes in response to specific cellular signals or environmental cues. In SCA, programmable gene circuits can be used to control the expression of fetal hemoglobin or other therapeutic genes in a precise, context-dependent manner, enhancing the efficacy and safety of the treatment.
2. **Safety Switches**: Incorporating safety switches into gene-editing constructs allows for the controlled activation or deactivation of the gene-editing machinery. This innovation can mitigate potential adverse effects by enabling clinicians to turn off the gene-editing process if unintended consequences arise, thereby improving the overall safety profile of the therapy.

Ethical and Socioeconomic Considerations

Addressing the ethical and socioeconomic challenges associated with gene-editing therapies is a crucial aspect of advancing the field.

1. **Equitable Access**: Ensuring that these cutting-edge treatments are accessible to all patients, regardless of socio-economic status, is essential. This involves developing cost-effective manufacturing processes, creating scalable delivery systems, and implementing policies that promote equitable distribution.
2. **Informed Consent and Public Engagement**: Engaging with the public and ensuring informed consent are vital for the ethical deployment of gene-editing therapies. Clear communication about the risks, benefits, and long-term implications of gene-editing treatments can foster public trust and support for these innovative therapies.

Conclusion

Telomere dynamics play a critical role in the pathophysiology of sickle cell anemia (SCA), influencing both disease progression and the molecular aging process. The accelerated telomere shortening observed in SCA patients, driven by oxidative stress, chronic inflammation, and DNA damage, contributes to the severity of the disease and its complications. Shortened telomeres are associated with increased frequency of vaso-occlusive crises, severe anemia, and early onset of organ damage, making telomere length a potential prognostic marker for disease severity and progression. Interventions

aimed at reducing oxidative stress and inflammation, enhancing DNA repair mechanisms, and potentially activating telomerase could help preserve telomere length and improve clinical outcomes for SCA patients. Additionally, monitoring telomere length could provide valuable insights into disease progression and aid in the personalization of treatment plans.

Abbreviations

SCA, Sickle Cell Anemia; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype.

Disclosure

The authors report no conflicts of interest in this work.

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CASE REPORT

Secondary Polycythemia May Be an Early Clinical Manifestation of Multiple Myeloma: A Case Report

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Abstract: Multiple myeloma (MM) is a malignancy of plasma cells that can cause anemia due to renal failure and bone marrow failure. Secondary polycythemia (SE) is a clinically rare disease that involves the overproduction of red blood cells. To our knowledge, the association of multiple myeloma and polycythemia has been reported, but the association of SE and multiple myeloma is rare and has been infrequently reported in literature. In contrast to anemia, the presence of polycythemia in multiple myeloma patients is a rare finding. A patient of IgA-λ multiple myeloma with secondary erythrocytosis recently admitted to our department is now reported as follows and relevant literature is reviewed to improve clinicians' awareness of such rare comorbidities.

Keywords: multiple myeloma, secondary erythrocytosis

Introduction

Multiple myeloma (MM) is a malignant disease with abnormal proliferation of clonal plasma cells and is the second most common malignancy in the blood system.[1,2] Globally, an estimated 588,161 individuals are diagnosed with MM each year, at a median age of 70 years.[3,4] Erythrocytosis encompasses some diseases characterized by increased circulating red blood cells (RBCs), which can be classified as relative, primary and secondary polycythemia. Secondary erycytosis (SE) can be caused by a variety of causes, including kidney tumors, other kidney diseases and inappropriate erythropoietin production, but is rarely associated with oxygen transport defects, usually by abnormal haemoglobin (Hbs) with increased oxygen affinity. Most of these patients are in generally good health, but RBCs and hemoglobin levels in the blood are higher than normal.[5] Case reports of MM and SE are increasing, and Lee et al suggested that SE may be an early clinical manifestation of plasma cell tumors.[6] Therefore, clinicians should pay attention to this patient group and strive to clarify the correlation between these two diseases soon, so as to better serve the patients. Here, we report a case of MM coexisting with SE and an attempt to analyze the relationship between these two coexisting diseases.

Case Report

A 75-year-old retired man was admitted to the hospital with erythrocytosis detected during follow-up of cerebral arteriosclerosis. Physical examination after admission revealed Sanguineous appearance, but there were no other positive sign. The blood oxygen saturation measured in the no-oxygen inhalation state was 96% (normal range 95–100%). He had hypertension and cerebral arteriosclerosis which were controlled regularly by long-term oral treatment of aspirin and atorvastatin calcium tablets, and no vascular events before. He had no previous smoking history and had never lived at high altitude. He complained of no dyspnea, orthopnea and paroxysmal nocturnal dyspnea. There was no family history of polycythemia or any myeloproliferative disorder. We performed laboratory tests after the patient's admission (Table 1). Further examination revealed that IgA-

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Table 1 Initial Laboratory Test Data

Variable	Admission	Reference Range
White Blood Cell (cells/L)	5.28 $\times 10^9$	4.5–10.8 $\times 10^9$
Differential Count (%)	N:45.0, L:45.2, M:8.3, E:1.1, B:0.4	N: 41.0–74.3, L: 18.3–47.9, M: 4.2–15.2, E: 0.4–8.0, B: 0.0–1.2
Hemoglobin (g/L)	175.0	120–160
Hematocrit (%)	54.70	40.0–50.0
Platelets (cells/L)	104x10^9	100–400x10^9
Mean Corpuscular Volume (fL)	101.0	81.0–98.0
Lactate Dehydrogenase (U/L)	210	125–274
Urea Nitrogen (mmol/L)	10.05	2.50–7.50
Calcium (mmol/L)	2.33	2.08–2.60
Creatinine (μmol/L)	143.2	40.0–120.0
Sodium (mmol/L)	142.0	135.0–145.0
Potassium (mmol/L)	3.67	3.50~5.50
Total Protein (g/L)	75.3	60.0–85.0
Albumin (g/L)	38.9	35.0–55.0
Globulin (g/L)	27.4	20.0–40.0
Albumin/Globulin	1.07	1.5–2.5
Total Bilirubin (μmol/L)	16.2	3.4–20.5
Indirect bilirubin (μmol/L)	8.2	0.0–18.0U/L
Direct bilirubin (μmol/L)	5.3	0.0–6.84 U/L
Alanine aminotransferase (U/L)	16.0	0.0–40.0
Aspartate amino transferase (U/L)	33.0	0.0–40.0
Total bile acids (umol/L)	39.0	0.0–25.0
Immunoglobulin A (g/L)	7.63	0.82–4.53
Immunoglobulin G (g/L)	8.88	7.51–15.60
Immunoglobulin M (g/L)	0.67	0.40–2.74
Complementary C3 (g/L)	0.65	0.79–1.52
Complementary C4 (g/L)	0.15	0.16–0.38
KAP light chain (g/L)	8.48	6.29–13.50
LAM light chain (g/L)	6.59	3.13–7.23
C-reactive protein (mg/L)	1.31	0.0–8.0
Hepatitis B surface antigen (IU/mL)	Positive; 30.57	Negative; < 0.05
Hepatitis B virus (IU/mL)	2.41E+03	< 2.00E+01
Beta-2 microglobulin (mg/L)	2.73	1.0–3.0

(*Continued*)

Table 1 (Continued).

Variable	Admission	Reference Range
Ferritin (ng/mL)	131.50	23.9–336.2
Haemopoietin (mIU/mL)	53.90	1.48–31.88
BCR::ABL1 P210/P190/P230	Negative	Negative
Mutation of JAK2/MPL/CALR/CSF3R	Negative	Negative
Serum free Kappa light chain (mg/L)	24.40	3.30–19.40
Serum free lambda light chain (mg/L)	68.82	5.71–26.30
Serum free kappa/free lambda (Ratio)	0.3545	0.2600–1.6500
Urinary free Kappa light chain (mg/L)	125.97	1.17–86.46
Urinary free lambda light chain (mg/L)	41.56	0.27–15.21
Urinary free kappa/free lambda (Ratio)	3.0310	1.8300–14.2600

λ was monoclonal with a M-spike of 5.65 g/L. The abdominal and urinary color examination showed no enlargement of the liver, spleen, kidney, and lymph nodes.Furthermore, a skeletal examination and magnetic resonance imaging of the spine showed no bone destruction. Bone marrow smear revealed 5.2% primary plasma cells (Figure 1). Flow cytometry further confirmed positive for λ chain, CD38 and CD138 in plasma cells (DxFLEX flow cytometer; Beckman Coulter, Inc). Bone marrow biopsy suggested the hyperplasia of nucleated cells in bone marrow is roughly normal (about 40% hematopoietic area); the granule/red ratio is roughly normal (Figure 2). Immunohistochemistry is shown CD138 (+), MPO (+), E-cad multicluster

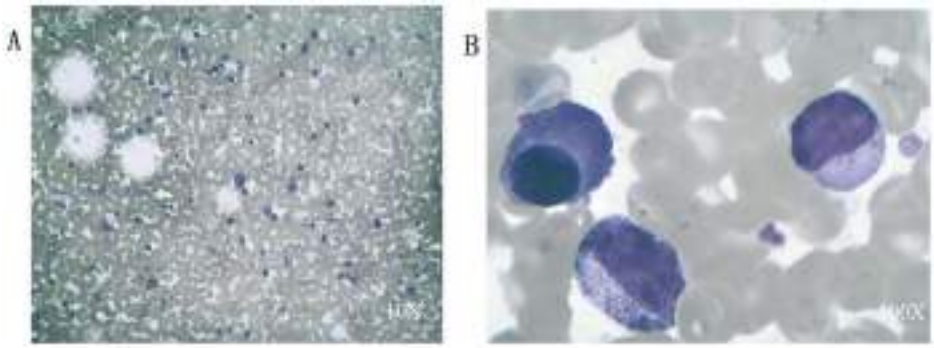

Figure 1 At the time of the MM diagnosis, the bone marrow was as above. (**A**) x10 magnification; (**B**) x100 magnification.

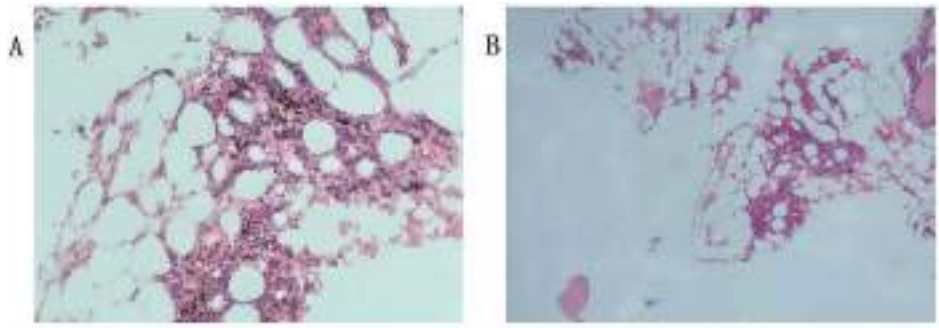

Figure 2 Bone marrow biopsy Immunohistochemistry: CD34 small vessel (+), occasional (+); CD11+ 17 occasional (+); CD61 megakaryocytes (+), sporadic (+); CD3 less (+); CD138 small cluster (+); MPO multiple (+); E-cad multicluster (+).

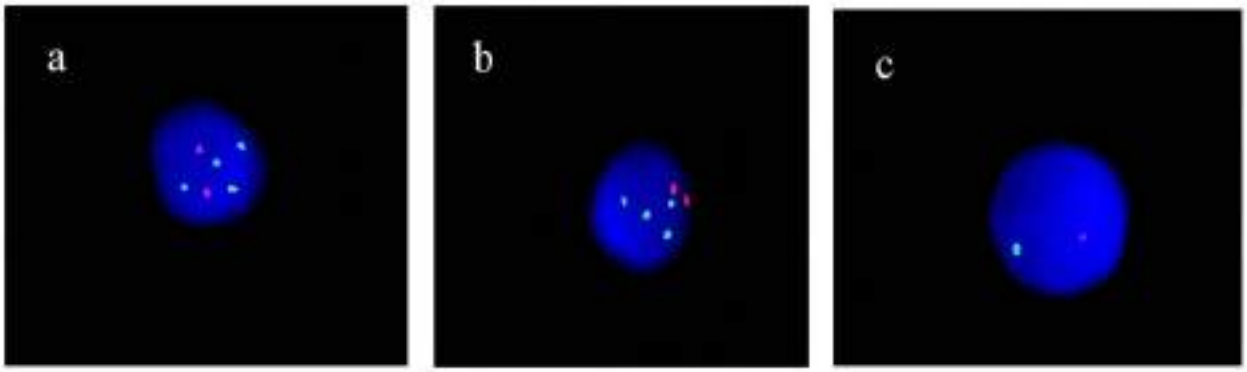

Figure 3 FISH: 1q21/1p32 (**a**), 13q14.3/(**b**), and 13q34 RB1(13q14) (**c**) genes are positive.

(+). Cytogenetic analysis revealed a normal female karyotype of 46 XX, while the mutation gene suggest that 1q21/1p32 13q14.3/13q34 RB1(13q14) genes are positive (Figure 3). Therefore, the patient was diagnosed with MM (IgA-λ DS Ia, ISS I, R-ISS I) (Durie-Salmon, DS; International Staging System, ISS; Revised International Staging System, R-ISS). He refused the associated treatment for SE (red blood cells were removed by apheresis), and now he is still asymptomatic.

Discussion

MM is a plasma cell monoclonal disorder derived from differentiated lymphoid B cells that causes uncontrolled growth, destructive bone lesions, kidney damage, anemia, and hypercalcemia. In contrast to anemia, the presence of polycythemia in patients with MM is rare, with only a few cases reported in the last 70 years.[7] In this patient, the concomitant polycythemia disappeared after myeloma treatment,[6–8] which may be related to the reduction of monoclonal light chain in the renal tubules.

Based on the etiology, erythrocytosis can be classified as polycythemia vera (PV), SE, and idiopathic polycythemia (IE).[9] The differential diagnosis of secondary polycythemia is extensive, but the most common etiology is chronic hypoxia.[10,11] Chronic hypoxia causing secondary polyred cell disease may be associated with multiple factors such as smoking, lung disease, living at high altitude, carbon monoxide exposure, hemoglobinopathy and sleep apnea.[7] After detailed history inquiry and examination, our patient quickly excluded the above reasons. The compensatory increase of erythropoietin (EPO) caused by various hypoxia, the increase of pathological EPO production and the increase of exogenous EPO production. EPO is composed of 165 amino acid residues, and its gene is located in the long arm 22 region of chromosome. The main function of EPO is to promote the proliferation, differentiation of late erythroid progenitors and their maturation, accelerate the release of reticulocytes and enhance the activity of antioxidant enzymes on the erythrocyte membrane.[12] It has been reported that SE of various reasons is related to the increase of EPO level.[13] The decrease of EPO level supports the diagnosis of PV, and the increase of EPO level is mostly SE.[14] In particular, under the negative condition of the JAK2 V617F mutation, EPO level is of great value in distinguishing PV from SE. This patient had a monoclonal immunoglobulin and 5.2% mature plasma cells, with occasional deformed nucleoplasma cell myeloid image, which was considered as focal onset of multiple myeloma lines. According to the diagnostic criteria of MM, the patient was diagnosis with MM, but no anemia occurred. In contrast, it showed increased RBCs from the currently known chronic bone marrow. As the results of genetic testing for proliferative tumors shown that, no mutations were detected in the JAK2-V617F gene, the JAK2 gene exons 12~15, the MPL gene, the CALR gene or exons, so polycythemia vera can be excluded. Polycythaemia caused by EPO-secreted tumors (e g, intracranial hemangioblastoma, hepatocellular carcinoma, renal cell carcinoma) was also ruled out by imaging studies. After a comprehensive systematic evaluation, the cause of polycythemia in this patient has not been clarified. We speculate that there may be an association between polycythemia and multiple myeloma in this patient.

The co-existence of polycythemia and multiple myeloma is rare, and some mechanisms may explain the association: ① Dominant or subclinical renal injury caused by monoclonal light chains in the renal tubules can lead to local hypoxia

and increased EPO generation.[8] Under normal oxygen tension, HIF-alfa is hydroxylated by EGLN 1 (PHD 2) and is targeted by VHL for ubiquitin-mediated degradation, resulting in the nonproduction of EPO.[15] Under hypoxia, the hydroxylase activity is inhibited. Thus, the EGLN 1 protein cannot hydroxylate HIF-alfa, thus enabling it to escape recognition and subsequent degradation by the VHL protein.[15] ② In recent years, it has been found that some tumors that do not originate from endocrine tissue can secrete one or more hormones and cause the corresponding excessive hormone excessive symptoms, which is called ectopic (source) hormone syndrome or associated hormone syndrome. Ectopic (source) erythropoietin or erythropoietin-like substances can lead to erythrocytosis.[16] In clinical practice, the appearance of this ectopic (source) hormone phenomenon can precede the symptoms of the tumor itself, which can be used as a diagnostic clue. ③ Other researchers have proposed that some tumors produce EPO or its predecessor,[17] which stimulates bone marrow hematopoietic tissue to produce more red blood cells, which requires further studies to confirm. ④ Notably, both elevated and decreased EPO levels have been reported in myeloma patients relative to the degree of anemia and renal dysfunction. Tumor cells (tumor polycythemia) have been described in uterine leiomyoma, hemangioblastoma, pheochromocytoma and so on.[7] Since malignant plasma cells do not produce EPO, this mechanism is unlikely to occur in polycythaemia-associated myeloma. ⑤The recently described TEMPI syndrome (Telangiectase, Erythrocytosis with elevation of erythropoietin, Monoclonal gammopathy, Perirenal effusion and Intrapulmonary shunting) may provide clues to the pathogenesis of polycythemia complicated with multiple myeloma.[18] Our patient was not eligible for the diagnosis of TEMPI syndrome owing to he did not have peri-renal effusion, telangiectasia and intrapulmonary shunt. However, polycythemia owing to exogenous EPO produced by myeloma cells is unlikely. His serum EPO levels were indeed elevated, and it could be speculated that, as with TEMPI syndrome, his erythrocytosis was due to monoclonal immunoglobulin increased HIF-1 α function and increased EPO production. Further studies may potentially clarify the association between MM and polycythemia.

Conclusion

MM incorporation of SE is rare, but the specific link between them is unclear. As the disease progresses, all patients eventually develop symptoms of anemia, which may be associated with high tumor burden, destruction of normal hematopoietic tissue in the bone marrow, or reduced generation of endogenous EPO. Therefore, we should closely monitor the condition of these patients, and need timely treatment once the patient has the treatment indication.

Ethics and Consent

The patient provided written informed consent for publication of this case report. This study is not required to obtain approval from the Ethics Committee of the First Affiliated Hospital for Jishou University.

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Disclosure

The authors declare that no conflict of interest exists in this work.

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Causes of Death and Mortality Trends in Individuals with Thalassemia in the United States, 1999-2020

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ORIGINAL RESEARCH

Causes of Death and Mortality Trends in Individuals with Thalassemia in the United States, 1999-2020

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Purpose: Our study aims to describe the mortality trends and disparities among individuals with thalassemia in the United States (US).

Patients and Methods: We used CDC WONDER database to calculate the age-adjusted mortality rates (AAMRs) per 1,000,000 individuals and used the Joinpoint Regression Program to measure the average annual percent change (AAPC). Subgroup evaluations were performed by sex, age, race, census region, and urbanization level.

Results: From 1999 to 2020, there were 2797 deaths relatd to thalassemia in the US. The AAMR of thalassemia-related death showed a decreasing trend from 0.50 (95% CI, 0.41–0.58) in 1999 to 0.48 (95% CI, 0.41–0.55) in 2020 with the AAPC of −1.42 (95% CI, −2.42, −0.42). Asians have the highest AAMR (1.34 [95% CI, 1.20–1.47]), followed by non-Hispanic Blacks (0.65 [95% CI, 0.59–0.71]), non-Hispanic Whites (0.32 [95% CI, 0.30–0.33]), and Hispanics (0.11 [95% CI, 0.08–0.14]). Cardiovascular disease remains the leading cause of death among individuals with thalassemia. The urban population has a higher AAMR than the rural population (0.43 [95% CI, 0.41–0.45] vs 0.29 [95% CI, 0.26–0.32]).

Conclusion: Our study calls for targeted interventions to address the racial and geographic disparities existed among individuals of thalassemia in the US.

Keywords: thalassemia, beta-thalassemia, alpha-thalassemia, epidemiology, mortality

Introduction

Thalassemias are a group of hereditary blood disorders resulting from decreased and/or defective α or β globin proteins in the hemoglobin as a result of gene mutation.[1] Hemoglobin is the primary oxygen carrier in the red blood cells. The dominant hemoglobin molecule in adults, known as HbA was made up of two α globin chains and two β globin chains. In thalassemia, the abnormal or low levels of α or β globin proteins disrupt the normal functioning of hemoglobin, leading to impaired erythropoiesis and anemia of varying severity among the affected individuals.[2]

Thalassemias are one of the most common causes of inherited anemia worldwide.[3] Approximately 56,100 infants are born with thalassemia annually, of whom more than half of them have transfusion-dependent β-thalassemia.[4] Generally, thalassemia was known to be highly prevalent in certain regions of the world, including Southeast Asia, the Mediterranean area, the Middle East, and Africa.[5] However, there is an increasing trend of prevalence in the other regions, namely North America and Northern Europe attributed to migrations.[6] In the United States, there was 7.5% increase in the prevalence of thalassemia in the last fifty years.[7]

Patients with thalassemia have varying presentation ranging from asymptomatic or mild anemia among patients with thalassemia trait to severe anemia requiring lifelong blood transfusion among thalassemia major patients. As a result of regular blood transfusion, patients may develop complications from iron deposition in multiple organs and tissues,

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including cardiac myocytes, liver, pancreas and other endocrine tissues. Over the last two decades, there have been some advances in the treatment of thalassemia, including the introduction of iron chelators, the use of luspatercept in reducing blood transfusion requirement,[8] and the recent approval of betibeglogene autotemcel gene therapy for transfusion-dependent beta-thalassemia major.[9]

With the increasing prevalence of thalassemia in the US, it remains uncertain of the mortality trends among individuals with thalassemia in the US. In addition, the advances in treatment does not always necessarily translate to improve outcomes at the population level due to the disparities existed across the population. Our study aimed to assess the causes of death and mortality trends among individuals with thalassemia, as well as the disparities among different demographics in the US.

Materials and Methods

Data Source

Centers for Disease Control and Prevention's Wide-Ranging Online Data for Epidemiologic Research (CDC WONDER) is a publicly available online database that contains public health data, including mortality data since the year 1999. We utilized the death certificate data in the CDC WONDER from 1999 to 2020 to examine the longitudinal trends of thalassemia-related mortality among the US population at all ages. Patients with thalassemia were identified using the code in the International Statistical Classification of Diseases and Related Health Problems-10th Revision (ICD-10). We excluded patients with unspecified ages and unspecified contributing causes of death. No prior institutional review approval is required as the data has been deidentified and is publicly available.

Using the CDC WONDER from 1999–2020, we first evaluated the demographics for mortality in thalassemia patients. We selected thalassemia as the contributing cause of death and measured the age-adjusted mortality rates, which were standardized to 2000 US census proportions.

The World Health Organization defines the contributing cause of death as any cause of death that is neither the direct, intervening, originating antecedent nor an underlying cause of death.[10] The demographic features were used to stratify the study population based on sex, age, race, and geographic region of residence at the time of death. This study methodology has been validated in similar research topics.[11,12]

Data Description

We utilized Microsoft Excel to organize the data into line graphs to show the overall AAMR trends of the individuals with thalassemia over the study period. We then grouped the data based on different demographic characteristics including sex, race, age, US census region, and urbanization level and used Microsoft Excel to exhibit the mortality trends among different groups. Joinpoint trend analysis was performed to determine the average annual percent change (AAPC) in the overall thalassemia-related mortality trends. Due to the smaller sample size of the study population when they were grouped under different demographic characteristics, percentage change from the period of 1999–2010 to 2011–2020 was calculated to compare the changes in the AAMR trends in these 2 periods.

Study Outcomes

The age-adjusted mortality rates (AAMRs) per 1,000,000 individuals were standardized for the year 2000 US census population. We compared the AAMRs in different sex, age, race, and geographic regions. For geographical variations, we cross-examined the AAMRs across different states, US census regions, and urbanization. The study population was categorized into urban (large central metro, large fringe metro, medium metro, and small metro counties) and rural (micropolitan non-metro and non-core non-metro counties) according to the 2013 US Census Classifications.[13]

Ethics Approval and Consent

Our study does not require institutional review approval as the population data is de-identified and publicly available.

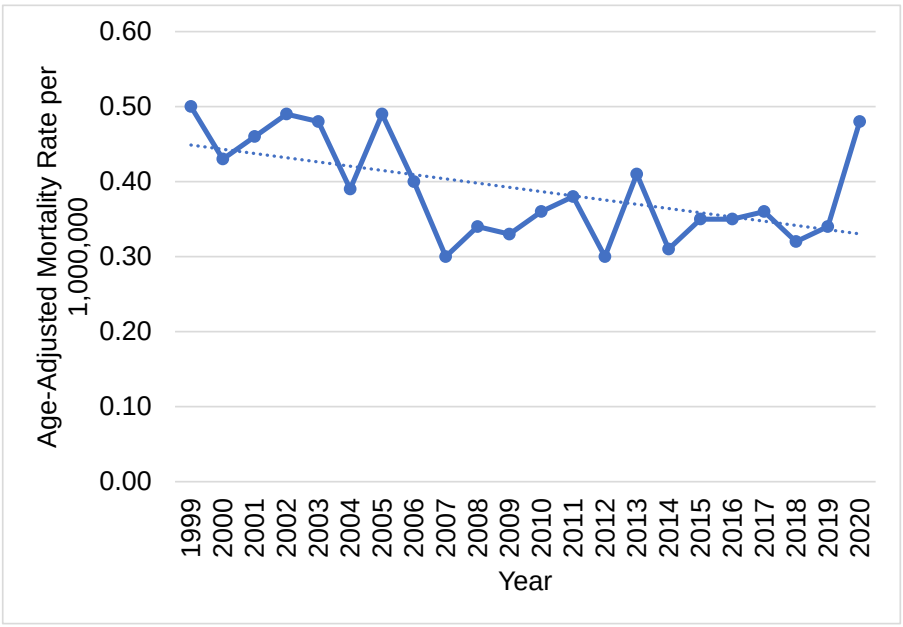
Results

From 1999 to 2020, there were 2797 deaths related to thalassemia, of which 1222 (43%) were male and 1575 (56%) were female. Out of 2797 deaths, there are 1857 (66%) deaths from thalassemia of unspecified types. When deaths from thalassemia of unspecified types were excluded, there were 700 (n=700/940, 74%) beta thalassemia deaths, 169 (n=169/940, 18%) alpha thalassemia deaths, and 71 (n= 71/940, 8%) thalassemia traits deaths. The baseline demographics of patients who met the inclusion criteria are shown in Table 1. Overall, the AAMRs decreased from 0.50 (95% CI, 0.41–0.58) per 1,000,000 individuals in 1999 to 0.34 (95% CI, 0.28–0.40) in 2019, then increased to 0.48 (95% CI, 0.41–0.55) in 2020, with the AAPC of −1.42 (95% CI, −2.42, −0.42) ($p < 0.01$) (Figure 1A).

Table 1 Demographic Characteristics of Thalassemia-Related Death in the United States, 1999–2020

Variable	N (%)	AAMR per 1,000,000 (95% CI)	Percentage Change from 1999–2010 to 2011–2020 (%)
Overall	2797 (100.00)	0.42 (0.40–0.43)	−7.50
Sex			
Male	1222 (43.69)	0.43 (0.40–0.45)	−7.32
Female	1575 (56.31)	0.40 (0.38–0.42)	−29.79
Race			
Non-Hispanic White	1820 (65.07)	0.32 (0.30–0.33)	−11.76
Non-Hispanic Black	489 (17.48)	0.65 (0.59–0.71)	−16.90
Asian or Pacific Islander	395 (14.12)	1.34 (1.20–1.47)	−21.85
Hispanic	73 (2.61)	0.11 (0.08–0.14)	−16.67
Age			
<20	206 (7.38)	0.03 (0.03–0.03)	0
20–39	265 (9.48)	0.04 (0.04–0.04)	−40.00
40–59	340 (12.16)	0.05 (0.05–0.05)	0.00
60–79	727 (26.00)	0.09 (1.77–1.85)	−20.00
≥80	1258 (44.99)	0.17 (0.17–0.17)	+5.88
US Census Region			
Northeast	660 (23.60)	0.46 (0.42–0.49)	−6.38
Midwest	541 (19.34)	0.34 (0.31–0.36)	−23.68
South	784 (28.03)	0.31 (0.28–0.33)	−20.00
West	812 (29.03)	0.53 (0.49–0.56)	−4.00
2013 Urbanization			
Urban	2435	0.43 (0.41–0.45)	−11.36
Rural	362	0.29 (0.26–0.32)	+6.90

Panel A



Panel B

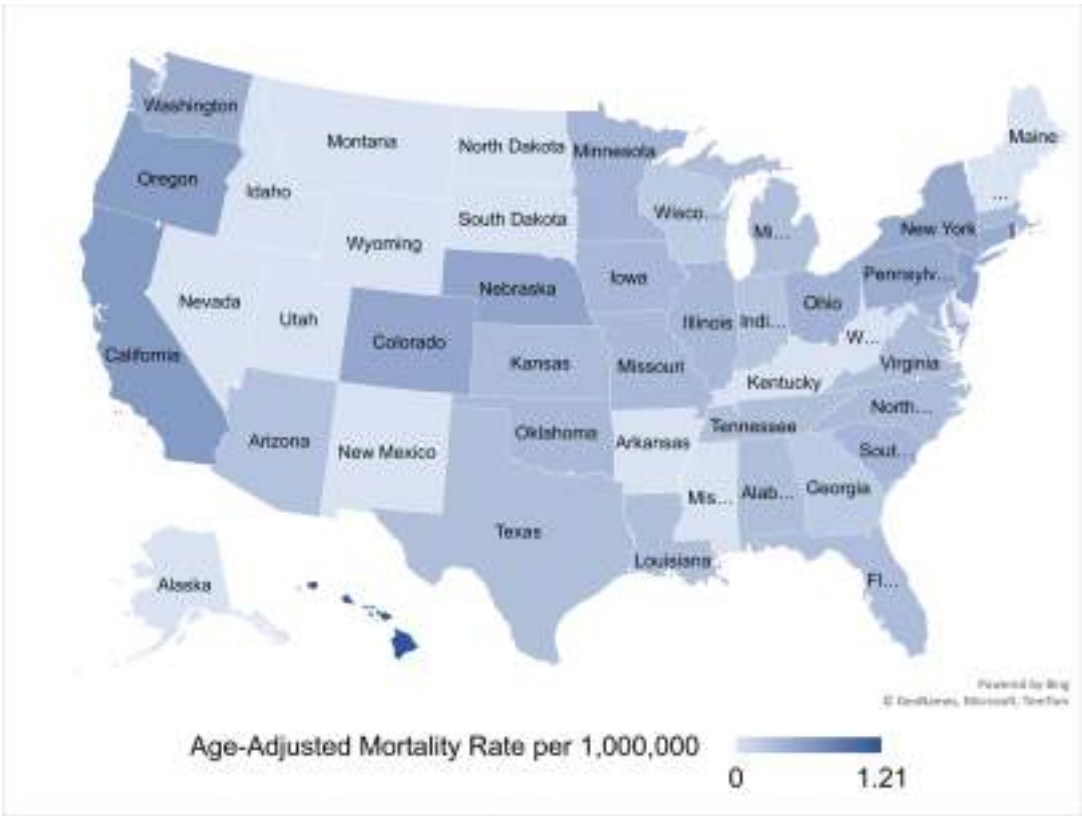


Figure 1 (**A**) Trend of age-adjusted mortality rate of thalassemia-related death between 1999 to 2020. (**B**) Overall age-adjusted mortality rate of thalassemia-related death stratified by states.

Causes of Death

Among individuals with thalassemia, the leading underlying causes of mortality when stratified by organ systems are cardiovascular (n=727, 26%), followed by hematological (n=579, 21%), malignancy (n=499, 18%), endocrine (n=176, 6%), respiratory (n=173, 6%), nervous (n=141, 5%), gastrointestinal (n=46, 5%), psychiatry (n=91, 3%), infection (n=84, 3%), and other systems (n=186, 7%) (Table 2). When the cardiovascular causes were further stratified, ischemic heart disease (n=309/727, 43%) was the most common reason, followed by hypertensive disease (n=83/727, 11%), cerebrovascular disease (n=48/727, 7%), aortic valve disease (n=30/727, 4%), cardiomyopathy (n=22/727, 3%), and atrial fibrillation and flutter (n=22/727, 3%). When the hematological causes were stratified, thalassemia (n=254/579, 43.9%) and sickle cell anemia (n=37/579, 6%). The three most common malignancies among individuals with thalassemia are lung or bronchus cancer (n=90/499, 18.4%), followed by myelodysplastic syndrome (n=42/499, 8.4%) and breast cancer (n=35/499, 7.0%).

Table 2 Underlying Causes of Death Among Individuals with Thalassemia in the United States, 1999–2020

Underlying Causes of Death by System	Number of deaths (%)	Most Common Causes within Each System (Number of deaths, %)
Cardiovascular	727 (26.0)	Ischemic heart disease (309, 42.5)
		Hypertensive diseases (83, 11.4)
		Cerebrovascular disease (48, 6.6)
		Aortic valve disease (30, 4.1)
		Cardiomyopathy (22, 3.0)
		Atrial fibrillation and flutter (22, 3.0)
Blood and blood-forming organs	579 (20.7)	Thalassemia (254, 43.9)
		Sickle cell anaemia (37, 6.4)
Neoplasm	499 (17.8)	Lung or Bronchus cancer (90, 18.4)
		Myelodysplastic syndrome (42, 8.41)
		Breast cancer (35, 7.0)
Endocrine	176 (6.3)	Disorders of iron metabolism (65, 36.9)
		Diabetes mellitus (44, 25.0)
Respiratory	173 (6.2)	Chronic obstructive pulmonary disease (76, 43.9)
		Pneumonia (21, 14.7)
Nervous system	141 (5.0)	Alzheimer's disease (87, 61.7)
		Parkinson's disease (21, 14.9)
Gastrointestinal	136 (4.9)	Liver cirrhosis (41, 30.1)
Mental	91 (3.3)	Dementia (71, 78.0)
Infection	101 (3.0)	Septicaemia (26, 25.7)
		COVID-19 (17, 16.8)
		Chronic viral hepatitis C (10, 9.9)
Others	169 (6.0)	Unspecified fall (14, 8.3)

Race and Age

Most of the deaths occurred in non-Hispanic Whites (n=1820, 65.1%), followed by non-Hispanic Blacks (n=489, 17.5%), Asians (n=395, 14.1%), and Hispanics (n=73, 2.61%). The highest AAMR was observed among Asians (1.34 [95%, 1.20–1.47] per 1,000,000 individuals), followed by non-Hispanic Blacks (0.65 [95% CI, 0.59–0.71] per 1,000,000 individuals), non-Hispanic Whites (0.32 [95% CI, 0.30–0.33] per 1,000,000 individuals), and Hispanics (0.11 [95% CI, 0.08–0.14] per 1,000,000 individuals) (Table 1).

Our study shows that the AAMR of patients with thalassemia increased with age. The highest AAMR was observed among patients aged 80 and above (0.17 [95% CI, 0.17–0.17] per 1,000,000 individuals), followed by patients aged 60–79 years (0.09 [95% CI, 0.09–0.09] per 1,000,000 individuals), patients aged 40–59 years (0.05 [95% CI, 0.05–0.05] per 1,000,000 individuals), patients aged 20–39 years (0.04 [95% CI, 0.04–0.04] per 1,000,000 individuals), and patients aged <20 years (0.03 [95% CI, 0.03–0.03] per 1,000,000 individuals).

Geographic Regions

When stratified by state, California had the largest percentage of deaths of individuals with thalassemia (n=487, 17.4%), followed by New York (n=221, 7.9%), and Florida (n=175, n=6.3%). The highest AAMR was observed in Hawaii (1.21 [95% CI, 0.86–1.65]) per 1,000,000 individuals), followed by Rhode Island (0.90 [95% CI, 0.58–1.32]) per 1,000,000 individuals), and California (0.61 [95% CI, 0.55–0.66]) per 1,000,000 individuals) (Figure 1B).

By US census region, the West recorded the highest AAMR at 0.53 (95% CI, 0.49–0.56) per 1,000,000 individuals, whereas the South recorded the lowest AAMR at 0.31 (95% CI, 0.28–0.33) per 1,000,000 individuals. The smallest decrease in AAMR was also observed in the West at −4.00% and the greatest decrease in AAMR was observed in the Midwest.

In terms of urbanization, the urban population had a higher AAMR compared to the rural population (0.43 [95% CI, 0.41–0.45] per 1,000,000 individuals vs 0.29 [95% CI, 0.26–0.32] per 1,000,000 individuals). Despite having a lower AAMR compared to the urban population, the rural population exhibited an increasing trend in the AAMR (+6.90%). On the contrary, the urban population exhibited a decreasing trend of AAMR from 1999–2010 to 2011–2020 (−11.36%).

Discussion

Our study provides important insights into the trends for thalassemia-related mortality in the US. Our study shows that (1) there is a decreasing trend in thalassemia-related mortality; (2) cardiovascular cause is the most common underlying cause of death among individuals with thalassemia; (3) Asians had the highest AAMR compared to other races; (4) urban population and the West had a higher AAMR compared to their counterparts.

Our study demonstrates a decreasing trend of thalassemia-related mortality from 1999 to 2019, and an increasing trend from 2019 to 2020. The initial decreasing trend of mortality rate could be multifactorial. First, alpha- and beta-thalassemia were often detected in the universal newborn screening programs that initially aimed to screen for sickle cell disease since 2006.[14,15] Besides, the approval of iron chelators, namely deferasirox and deferiprone in the US in 2005 and 2011 respectively, delays the development of iron-induced cardiac toxicity which may lead to heart failure and arrhythmia.[16,17] As cardiovascular causes are the most common cause of death among patients with thalassemia, the declining death rate among patients with thalassemia can also be related to the declining death rate attributed to ischemic heart disease in the last two decades[18] as a result of the increased availability of preventive interventions and advancements in therapy.[19] We propose that the later upward trend in mortality may be attributed to the impacts of the COVID-19 pandemic.

Our study found that cardiovascular cause, hematological cause, and malignancy are the three most common causes of death, comprised of more than half of the deaths among individuals with thalassemia. It was observed that cardiovascular causes are the most common cause of death among individuals with thalassemia, similar to the general population.[20] This is consistent with the existing studies which show heart diseases are the leading causes of death for both thalassemia major patients[21,22] and non-transfusion-dependent thalassemia.[23] There are multiple explanations for this. Individuals with thalassemia were similarly burdened by cardiovascular risk factors eg, hypertension, dyslipidemia, and diabetes mellitus like the general population.[24] Besides, individuals with transfusion-dependent thalassemia are

prone to develop cardiac complications from iron overload, including sudden cardiac death, arrhythmia, and heart failure.[25] Similar to the general population, lung and bronchus cancer and breast cancer are the leading causes of cancer death in our study population.[26] Myelodysplastic syndrome is found to be one of the common causes of cancer death among individuals with thalassemia, which is consistent to a study done by Chung et al.[27] It was suggested that iron accumulation from chronic blood transfusion can induce oxidative stress and DNA damage, contributing to the pathogenesis of MDS.[28]

Besides, our study demonstrates that Asians had the highest AAMR compared to other racial groups. This can be explained by the higher prevalence of thalassemia among the Asians. A previous study found that Asian patients made up more than half of the thalassemia population in North America.[29] Asians are predisposed to have thalassemia due to their genetic predisposition such as the deletions of HBA1 or HBA2.[30,31] The higher mortality in certain geographic regions such as the West and the urban areas can be related to the immigration patterns of Asians to the United States. The higher mortality in the urban population can also be related to the fact that almost one-third of the Asian immigrants lived in the metropolitan areas from 2015 to 2019.[32] The highest mortality was observed in the West, mainly in California. This may be due to the fact that almost half of the US Asian population resides in the West, with about 30% of them in California.[33]

There are a few limitations in our study. First, the database consists of no clinical and imaging data, therefore we are unable to further characterize the clinical status of the population. Besides, the database has no information at individual levels, such as comorbidity burden, duration of diseases, medical treatments, or prior intervention, which are important confounders for mortality. Despite these limitations, our study sufficiently demonstrates the demographic and temporal mortality trend of patients with thalassemia. It provides valuable insights into the need to improve the outcome of patients with thalassemia.

Conclusion

Current management of thalassemia includes transfusion therapy, use of Luspatercept in reducing transfusion requirement, iron chelation, and curative options like hematopoietic stem cell transplantation (HSCT).[34] Population-based study is essential to measure the outcomes of the latest treatment. Besides, our study highlights cardiovascular causes as the predominant cause of mortality among patients with thalassemia. This underscores the need for targeted cardiovascular monitoring and management in this patient population. Although the mortality rate among individuals with thalassemia declined over the years, the disparities existed across different races and geographic regions demonstrated in our study suggested that targeted interventions are required to address the disparities in the mortality trends among the individuals of thalassemia in the US.

Data Sharing and Data Accessibility

The datasets generated during and/or assessed during the current study are available in the CDC WONDER, https://wonder.cdc.gov/

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Disclosure

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ORIGINAL RESEARCH

Clinical and Biomarker Characteristic of Lymphoma Patients in Hasan Sadikin Lymphoma Registry

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Background: No specific data have been systematically collected regarding lymphoma patient characteristics, while non-Hodgkin lymphoma (NHL) is identified as the 7th most common cancer and Hodgkin lymphoma (HL) is the 28th. Inflammation plays an important role in the pathogenesis and progression of lymphoma. Malnutrition is an adverse prognostic factor in lymphoma. Systemic Inflammatory Index (SII), Prognostic Nutritional Index (PNI), and Advanced Lung Cancer Inflammation Index (ALI) were biomarkers depicting inflammation and nutritional status. This study aims to describe the clinical and biomarker characteristics of both HL and NHL patients.

Methods: This descriptive study used a cross-sectional design, and data were collected from Hasan Sadikin Hospital lymphoma registry from January 2020 to November 2023. Demographic, staging, and histopathological data were extracted. Three biomarkers were evaluated. Survival curves were drawn using Kaplan–Meier curve analysis, and the log rank test was used for comparison of survival between early and advanced stage.

Results: A total of 271 patients were recruited as participants, and the majority (80.5%) had NHL, with diffuse large B-cell lymphoma (DLBCL) being the most common histopathological type (50.5%). Early disease was observed in two-thirds of patients, and low-risk International Prognostic Index (IPI) score was the most common prognostic score found (95%). SII was slightly higher in early compared to advanced stages. Treatment response was evaluated from 101 patients, and complete response was observed in 44.5%. Two-year overall survival (OS) was 93.1%, with median survival 22.7 (95% CI 21.9–23.5) months. In early stage, the median survival was slightly longer than in advanced stage [23.0 (95% CI 22.2–23.8) vs 21.6 (95% CI 19.3–23.8) months, P=0.09].

Conclusion: Hodgkin lymphoma and DLBCL had similar clinical and biomarker characteristics. There were slight differences between the three biomarkers SII, ALI, and PNI based on the disease stage. Almost all patients still survived at 2-year follow-up.

Keywords: lymphoma, non-Hodgkin lymphoma, Hodgkin lymphoma, registry

Background

Lymphoma is a malignancy affecting lymphocytes and lymphoid tissues, particularly the lymph nodes and associated organs such as the spleen. These cancers are classified based on cellular origin, with molecular diagnostics playing an increasingly significant role. Currently, more than 80 different entities are recognized in this classification system.[1]

In 2020, 83,087 cases of Hodgkin lymphoma (HL) constituted 0.4% of newly reported cancer occurrences and accounted for 0.2% of cancer-related deaths worldwide. The global age-standardized incidence rate for HL was 0.98 per 100,000 people.[2,3] Meanwhile, non-Hodgkin lymphoma (NHL) imposes a significant global burden, with the incidence increasing rapidly in recent decades. In 2020, NHL was identified as the 11th most frequently diagnosed cancer, with

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nearly 545,000 new cases and ranking as the 11th leading cause of death, resulting in approximately 260,000 fatalities. According to GLOBOCAN data, NHL ranks as the 7th most common cancer in Indonesia, while HL is at the 28th position.[2]

The etiology and development of lymphoma are significantly influenced by inflammation.[3] According to Park et al, malnutrition is a poor predictive factor for lymphoma.[4] Biomarkers that represented inflammation and nutritional status included Systemic Inflammatory Index (SII), Prognostic Nutritional Index (PNI), and Advanced Lung Cancer Inflammation Index (ALI).[5]

There has been no systematic collection of specific data on lymphoma patient characteristics. By assembling a standardized minimum dataset that progressively incorporates patient-reported results, registries prove highly beneficial, particularly for diseases or interventions posing challenges for clinical trials. Even in large referral centers where patient numbers may be limited, registries play a crucial role, providing a means to detect variations in medical practices. Additionally, registries function as efficient platforms for executing observational studies and interventional trials, facilitating the establishment of optimal management methods and enabling health economics analyses through the use of "real world" data.[6]

The purpose of this study is to characterize the clinical features and biomarkers of inflammation and nutritional status of lymphoma patients in our hospital.

Method

A cross-sectional design was applied in this descriptive study, using lymphoma registry data collected from the Oncology clinic at Dr Hasan Sadikin General Hospital in Bandung from January 2020 to November 2023. Participants were selected through a total sampling method, with the following inclusion criteria: patients suspected or diagnosed with lymphoma, confirmed by histopathology, and engaged in a minimum of one chemotherapy cycle. Furthermore, the variables explored were age, gender, sex, age at diagnosis, year of diagnosis, baseline hematologic profile, histological type, Ann Arbor stage, and treatment modalities. Death confirmation relied specifically on medical records from Hasan Sadikin Hospital. Additionally, multiple information-gathering steps were carried out, including raw data collection, data abstraction and coding, initial verification, data input, secondary verification, data analysis, and reporting. Patient lists were obtained from medical records and the hospital information system, filtered for duplication, hand-searched for correspondence, and subjected to data abstraction and coding. The demographic, diagnostic, staging, and histopathological data were collected.

The following formulas were utilized to calculate these values: NLR (neutrophil lymphocyte ratio: peripheral blood levels of absolute neutrophil count/absolute lymphocyte count; ALI: body mass index (BMI) × blood albumin level (g/dL)/NLR; PNI: 10 × serum albumin (g/dL) + 0.005 × total lymphocyte count/mm^3; SII: platelet count × NLR.[5] Unpaired *t* testing or Mann–Whitney *U* testing was used to compare continuous variables, while categorical data were compared using χ^2 testing or Fisher exact testing to differentiate characteristics between HL and DLBCL patients. Two-year survival curves were drawn using Kaplan–Meier curve analysis, and we used the log rank test for comparison between early and advanced stage. A P value <0.05 was considered statistically significant.

All the data were analyzed using Microsoft Excel and SPSS version 25.0.

The permission to conduct this study was obtained under the Ethical Approval Number DP.04.03/D.XIV.2.2.1/197/2024.

Result

A total of 271 patients with lymphoma, of whom 161 (63.1%) were males, were included in this study, and the demographic characteristics were assessed as presented in Table 1. Approximately two-thirds of participants were 60 years old or younger, with a median BMI in normal limits before treatment. Only 11.4% were underweight, and hematological parameters observed before treatment remained in normal limits. The majority (80.5%) had NHL, with diffuse large B-cell lymphoma (DLBCL) being the most common histopathological type. T-cell lymphoma was found in only 3/271 (1.1%) patients. Early-stage disease (stages I and II) was observed in two-thirds of patients, and most had good performance status (ECOG 0 and 1). The low-risk IPI score was the most common prognostic score found, and approximately half of patients received an R-CHOP or CHOP chemotherapy regimen. Four biomarkers including SII, PNI, ALI, and LMR/LDH ratio showed slight differences between early and advanced stages.

Table 1 Clinical Characteristics

Characteristic	
Participants (*n*)	271
Demographic Characteristics	
Age, median (IQR), years	50 (34–62)
≤60 Years (*n*, %)	198 (73.1)
>60 Years (*n*, %)	73 (26.9)
Male sex (*n*, %)	161 (63.1)
Clinical Characteristics	
Body Mass Index (BMI) before Treatment [Median (IQR), kg/m^2]	21.9 (19.8–24.6)
Underweight (BMI<18.5) (*n*, %)	31 (11.4)
Normoweight (BMI≥18.5) (*n*, %)	240 (88.6)
Hematological Parameters, Median (IQR)	
Hemoglobin (g/dL)	12.7 (11.0–14.1)
White blood cells (/mm^3)	7800 (6200–10,500)
Neutrophil count (/mm^3)	4896 (3560–6896)
Lymphocyte count (/mm^3)	1675 (1054–2420)
Platelets count (/mm^3)	323,500 (252,000–397,000)
Pathology Anatomy (*n*, %)	
Hodgkin lymphoma	53 (19.5)
Non-Hodgkin lymphoma	218 (80.5)
– Diffuse large B-cell lymphoma	137 (50.5)
– Follicular lymphoma	35 (12.9)
– Marginal zone lymphoma	11 (4.0)
– Mantle cell lymphoma	4 (1.5)
– T cell lymphoma	3 (1.1)
Stage	
– I	82 (30.2)
– II	131 (48.4)
– III	42 (15.5)
– IV	16 (5.9)
ECOG	
– 0	215 (79.3)
– 1	53 (19.5)
– 2	2 (0.8)
IPI Score	
Low risk (0–1)	258 (95.0)
Low intermediate risk (2)	13 (5.0)
High intermediate risk (3)	0
High risk (4–5)	0
Chemotherapy regimen	
– CHOP	113 (41.7)
– R-CHOP	115 (42.5)
– ABVD	43 (15.8)
Biomarker, Median (IQR)	
– SII	
Early stage	947,760 (537,762.5–1,681,826)
Advanced stage	854,031 (363,701.5–1,778,635.2)

(*Continued*)

Table 1 (Continued).

Characteristic	
– PNI	
Early stage	39.2 (10.1–47.4)
Advanced stage	40.3 (13.1–48.7)
– ALI	
Early stage	8.5 (1.7–32.5)
Advanced stage	14.4 (8.7–43.7)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; SII, Systemic Immune Inflammatory Index; PNI, Prognostic Nutritional Index; ALI, Advanced Lung Cancer Inflammation Index.

Table 2 shows the comparison of clinical characteristics between HL and DLBCL patients. There was no significant difference in demographic, clinical, and biomarker characteristics between the groups.

At the time of analysis, half of the patients had completed chemotherapy, with the majority achieving a complete response. Out of 271 patients, 56 (20.6%) failed to complete follow-up, and 14 (5.2%) deaths were recorded during treatment; patient's outcome are shown in Table 3.

Table 2 Clinical Characteristics Difference Between HL and DLBCL

Characteristic	HL n=53	DLBCL n=137	p
Demographic Characteristics			
Age, median (IQR), years	48 (37–60)	52 (40–63)	0.231
≤60 Years (*n*, %)	49 (92.4)	55 (40.2)	
>60 Years (*n*, %)	4 (7.6)	82 (59.8)	
Male sex (*n*, %)	26 (49.1)	87 (63.5)	
Clinical Characteristics			
Body Mass Index (BMI) Before Treatment [median (IQR), kg/m^2]	18.1 (17.6–25.8)	17.9 (17.6–28.7)	0.266
Underweight (BMI<18.5) (*n*, %)	9 (16.9)	15 (10.9)	
Normoweight (BMI≥18.5) (*n*, %)	44 (83.1)	122 (89.1)	
Hematological Parameters, Median (IQR)			
Hemoglobin (g/dL)	12.5 (10.6–14.0)	12.4 (11.0–14.1)	0.848
White blood cells (/mm^3)	7500 (5400–11,640)	7800 (6200–10,245)	0.679
Neutrophil count (/mm^3)	4480 (3027–6751)	4960 (3572–6890)	0.347
Lymphocyte count (/mm^3)	1596 (1015–2272)	1692 (1053–2558)	0.464
Platelet count (/mm^3)	341,000 (266,000–398,500)	328,000 (255,500–409,000)	0.959
Stage			
– Early	109 (79.5)		0.540
– Advanced	28 (20.5)		
ECOG	52 (98.1)		
– 0–1		137 (100)	1.000
– 2–3	1 (1.9)	0 (0)	
Biomarker, Median (IQR)			
SII	978,000 (520,791–2,290,962)	1,009,810 (514,948–1,677,870)	0.938
PNI	9.3 (5.6–12.9)	8.9 (6.3–14.1)	0.492
ALI	0.4 (0.0–1.7)	0.53 (0.0–1.44)	0.813

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; SII, Systemic Immune Inflammatory Index; PNI, Prognostic Nutritional Index; ALI, Advanced Lung Cancer Inflammation Index.

Table 3 Outcome of Treatment

Outcome	n (%)
Completed Chemotherapy	118 (43.5)
Chemotherapy Response [a](*n*=101)	
– CR	45/101 (44.5)
– PR	20/101 (19.8)
– SD	27/101 (26.7)
– PD	9/101 (8.9)
Drop out	56 (20.6)
Death before completed chemotherapy	14 (5.2)

Notes: [a]chemotherapy response could not be evaluated in 17 patients due to loss to follow-up.
Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

After 2 years of follow-up, we demonstrated a 2-year overall survival (OS) of 93.1% with median survival 22.7 (95% CI 21.9–23.5) months. In early stage, the 2-year OS and median survival were slightly better than in advanced stage [94.9% vs 85.7% and 23.0 (95% CI 22.2–23.8) months vs 21.6 (95% CI 19.3–23.8) months, $P = 0.09$] (Figure 1).

Discussion

Clinical and pathological features of 271 lymphoma cases in Hasan Sadikin General Hospital were assessed from 2020 to 2023 during this study. The patients were younger than the age reported for those with Western lymphoma, and most were not malnourished. Additionally, NHL was the dominant case found, with DLBCL being the most common type. In two-thirds of patients, the early disease stages I and II were observed, and the majority had good performance status. Moreover, the most prevalent predictive score discovered was a low-risk IPI Score. R-CHOP or CHOP regimens were administered to about half of the patient population who had finished chemotherapy, with the majority showing complete response.

The median age of lymphoma patients was 50 (IQR, 34–62) years, correlating with the range of 45–65 years among 834 participants reported by Dwianingsih et al in Yogyakarta, Indonesia, from 2010–2014.[7] In 203 DLBCL patients in Surabaya from 2015–2017, the mean age recorded was 51 ± 12.9.[8] In Singapore, 18 to 94 (mean 55.0±16.2) years was observed,[9] which was younger than the median 63 years reported during previous study by Mugnaini and Ghosh in the United States from 2009 to 2013. Similarly, in Australia and New Zealand, the median age of 64.3 (range, 52.1–73.5) years was identified.[10,11] The median age of those with NHL in Asia is significantly lower, compared to the population-based registration in Western countries. Risks for developing NHL include immunosuppression, a causal connection between infectious agents and

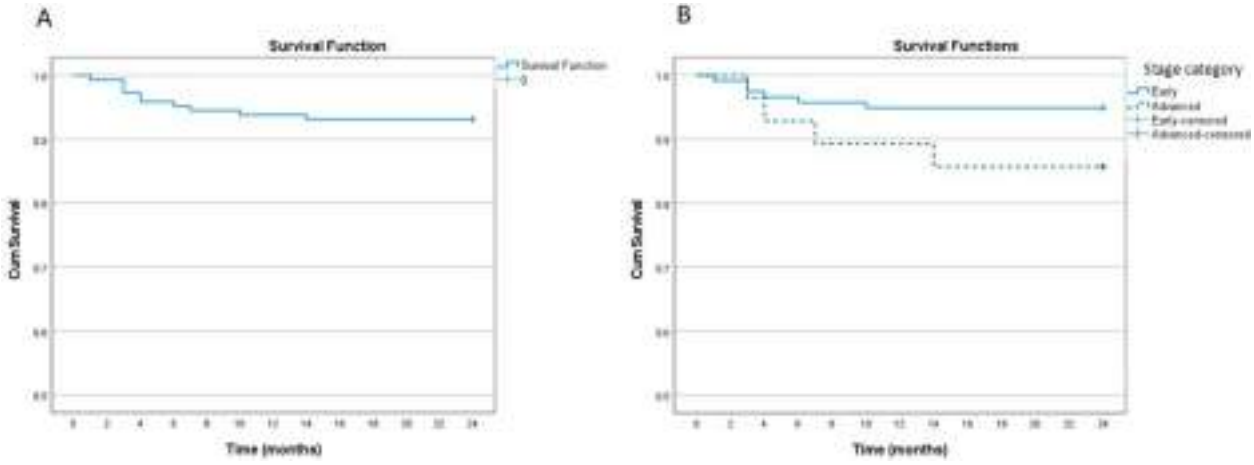

Figure 1 Survival curve for 145 patients in 2 years of follow-up. (**A**) All patients. (**B**) Early vs advanced stage.

lymphomagenesis. These have been determined, particularly for human T-cell leukemia/lymphoma virus type1 (HTLV-1), Epstein–Barr virus (EBV), *Helicobacter pylori* infections, and hepatitis C viruses (HCV), which are frequently detected in Asia.[12] These factors could be the potential reason for the younger age of lymphoma patients in Asia. The age group <60 years constituted 73.1% in this study, corresponding to previous investigations by Reksodiputro who reported NHL patients constituting 78% (117/153) from 2004–2005. Similarly, 78.8% (119/203) of individuals with DLBCL receiving R-CHOP chemotherapy during 2015–2017 in Surabaya[8,13] and 78.8% (598/791) in India were <60 years.[14]

The assessed patients were predominantly male (63.1%), comparable to the previous report of 60.8% and 61.3% of those with lymphoma in Jakarta and Yogyakarta being male, respectively.[7,13] In Singapore, the gender ratio of lymphoma patients among male and female was 1.3:1.[9] In Australia and New Zealand, 59.6% of the total population suffering from lymphoma was male.[9] Among DLBCL patients, Dilliawan reported 63.6% male in Surabaya,[8] while a 61.7–67.1% male gender distribution was found in India.[14,15] The precise reasons for the sex-based disparities in lymphoma occurrence are unknown, but immunological and hormonal abnormalities, body size, tumor biology, and different exposures to environmental contaminants have been suggested as the causes.[16,17]

Malnutrition, defined as BMI <18.5, was found in 11.4% of the lymphoma patients, which was lower than 47.4% observed during a previous study conducted in 2017.[18] The 47.4% value was higher than the BMI <18.5 reported in the US among only 1% of lymphoma patients.[19] This disparity can be caused by different socioeconomic status, and it is possible for tumor impact or body systemic reaction against the tumor to trigger high production of pro-inflammatory cytokines. An important clinical investigation for lymphoma stated the function of interleukin-6 (IL-6) in generating malnutrition in lymphoma.[20]

Lymphoma is broadly classified into HL (10%) and NHL (90%) pathological subtypes.[10] The distribution of these subtypes (NHL vs HL: 80.5% vs 19.5%) in Hasan Sadikin General Hospital registry is consistent with reports from another Indonesian region (Yogyakarta), India, and Australia.[7,11,14] Singapore had a slightly higher percentage of NHL patients compared to HL at 90.8% and 6.9%, respectively.[21] The most common lymphoma type detected among the patients in this study was DLBCL (50.5%), which correlated with a previous report of 53% DLBCL from 2004–2005 in Jakarta.[13] In other regions of Indonesia, DLBCL was found at a level of approximately 44.4%.[7] The proportion of DLBCL patients recorded in the registry was similar to 55% and 53.3% cases reported in India and Singapore, respectively.[9,14] However, it was higher than the proportion reported in Korea (31%), Japan (33%), and China (36%).[5,22,23] This might represent variations in genetic and environmental exposures among Asian communities.[9] Follicular lymphoma in 12.9% (35/271) patients was the second most common type identified, exceeding the 0.8% and 8.0%-11.5% found in Yogyakarta and Singapore, respectively.[7,9,21] However, the value was smaller compared to the 15.4% recorded in Australia,[11] suggesting that T cell lymphoma in this study (1.1%) was lower than the reports in Japan (10%).[23]

The majority of patients (78%) were in the early stages I and II, correlating to another study by Reksodiputro which reported early-stage disease among 68.97%.[13] These results contradicted a previous report of early-stage disease among 43.5% in India from January 2005 to December 2009, suggesting the possibility of differences between periods of data collection.[14] Based on ECOG values of 0–1, good performance status (PS) was found among 98.8% of patients, exceeding 86.7% reported by Reksodiputro, as well as the observed 69.9% in India and 88.8% in Australia.[11,13,14] These differences could be significantly influenced by subjective measures affecting PS assessment.

Most patients had a low-risk IPI score of 0–1 (95.0%), which was higher than the values reported in China (44.3%) and India (70%).[14,15,24,25] This discrepancy could be attributed to different lymphoma types included in this current study, while Nimmagadda studied only DLBCL patients.

The chemotherapy regimen mainly applied in this study was CHOP, while R-CHOP was provided to 42.5%. Similarly, a previous investigation identified CHOP as the most commonly administered (84%), and 42.7% of those suffering from DLBCL were treated using rituximab.[26] In Surabaya, Salma reported the provision of CHOP and R-CHOP for 79.48% and 20.52% of patients with NHL, respectively.[27] R-CHOP only was given in 42.5% due to the availability of rituximab in our government's health insurance which only approved this monoclonal antibody for DLBCL.

Our study found the median age of HL patients was 48 (IQR, 37–60) years. This was older than previous study in Taiwan that reported the median age of HL was 26 (range, 3–84) years.[28] In our DLBCL patients, the median age was 52 (IQR, 40–63) years. This finding was similar with previous finding that reported the median age of DLBCL patients in 57

(range, 7−85) years.[29] Our study did not find difference in clinical and biomarker characteristics between HL and DLBCL patients.

The chemotherapy responses were complete response (CR) in 44.5% of cases and partial response (PR) in 19.8%, which was similar to CR (52.38%), PR (26.19%), and minimal response (14.2%) reported among the Indonesian population in 2011.[11] Based on the data from 2011–2015 in Surabaya, 51.3% of NHL patients had CR, and 28.2% achieved PR.[27] These response rates were lower compared to previous external studies conducted, such as in China, where the overall response rate (CR+PR) was 83.3%, and 67 (11.8%) patients achieved stable disease (SD) or suffered from progressive disease (PD) after receiving CHOP-like or R-CHOP-like regimen.[26]

Exploration of simple biomarkers in lymphoma has become an essential area of study. Previous investigations evaluated SII, PNI, and ALI among DLBCL patients, identifying slightly higher SII in early compared to advanced disease stages. However, in the early stages, both PNI and ALI showed slightly decreased levels. ALI and PNI may serve as easily available markers to predict clinical results in DLBCL patients, while SII can predict overall survival (OS) only in univariate analysis.[25] Our study found that, compared with early stage, SII was higher, whereas PNI and ALI were lower in advanced stage. This reflected that in advanced stage of lymphoma there were higher inflammation and lower nutritional status. The systemic immune-inflammation index (SII), based on neutrophil, platelet, and lymphocyte counts, is a prognostic biomarker and in some solid cancers may reflect the inflammatory status and tumor activity. Prognostic nutrition index (PNI), a variable based on serum albumin concentration and total lymphocyte count in peripheral blood, is a scoring system that reflects the nutritional status and immune status of patients.[30] The Advanced Lung Cancer Inflammation Index (ALI) has been demonstrated to be a prognostic factor of survival in some solid cancers and lymphoma.[31] Serum albumin, as an important nutritional indicator, plays an important role in improving the body's immunity, inflammatory state, and anti-tumor activity.[5]

At the time of analysis, 2-year OS was 93.1%. The 2-year OS in early stage was higher than in the advanced stage group (94.9% vs 85.7%). Previous study reported estimated 2-year OS of 61.6% (95% CI 54.1–68.2%) in DLBCL patients.[32] This difference could be caused by subject's variation between the studies: we included all lymphoma patients, while previous study only evaluates DLBCL.

Study Limitations

Some of the strengths and limitations associated with this study are described as follows. This is the first investigation conducted on the characteristics and biomarkers of lymphoma patients, including treatment response, in Hasan Sadikin General Hospital over a three-year period. However, due to the retrospective nature of this study, there is a possibility that the information extracted from the case charts may not be entirely accurate or comprehensive. A substantial portion of the patient population was lost to follow-up, and not all chemotherapy response was evaluated using appropriate imaging. Despite these limitations, the analysis performed is expected to provide a significant contribution to the literature of lymphoma in Indonesia.

Conclusion

In conclusion, this study showed similarities between demographic characteristics of lymphoma patients with features reported from many developing nations, but differences were observed in the distribution of PS and IPI-score. The chemotherapy response was comparable with the rates found in other investigations, while the biomarkers SII, PNI, and ALI were essential parameters requiring exploration. At 2-year follow up, almost all patients were still alive. Subsequently, a more robust system of lymphoma registry should be effectively established. The registry could be used as a platform for observational studies and clinical trials, enabling effective, long-term follow-up.

Ethics Statement

This study was approved by the ethics committees of the Hasan Sadikin General Hospital and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from patients.

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.

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CASE REPORT

A Case of Pernicious Anemia with Concurrent Beta-Thalassemia Minor

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Abstract: Vitamin B_{12} is essential for various bodily functions, and its deficiency may cause hematological manifestations. We report a case of a previously healthy 65-year-old female who was admitted to our hospital with reduced sense of taste and painful tongue. The serum level of vitamin B_{12} was decreased. However, her complete blood count did not show any evidence of macrocytosis, instead, her mean corpuscular volume was low. Gene sequencing indicated an β-thalassemia minor and that probably masked the megaloblastic features of vitamin B_{12} deficiency.

Keywords: pernicious anemia, autoimmune gastritis, thalassemia, glossitis

A 65-year-old woman presented with reduced sense of taste, painful tongue, decreased appetite, fatigue and dizziness that had developed 10 months earlier. She had been confused for a long time because of tasteless eating and loss of appetite due to the reduced sense of taste. She referred to the outpatient department of stomatology several times without definite diagnosis. She had no signs of infection or bleeding, no family history of hematologic disorders, and a medical history significant only for hypertension. Her bowel movements were normal with no evidence of blood. There was no surgical history or alcohol consumption.

On physical examination, the patient appeared pale but fully oriented, with stable vital signs and no fever. She had a smooth, red tongue without lingual papillae, suggestive of glossitis (Figure 1A). She had no palpable hepatosplenomegaly or lymphadenopathy, and no deficits were found on neurologic examination.

Laboratory detection indicated normocytic anemia (hemoglobin 78 g/L, MCV 85.5 fL [normal range 82–100], MCH 27.6 pg [normal range 27–34], MCHC 322 g/L [normal range 316–354], RDW 27.5%, platelets 361×10^9 /L, and white blood cells 3.52×10^9 /L). A complete blood count conducted six months prior did not show any irregularities. Additionally, high-sensitivity troponin levels and electrocardiography results were unremarkable, and renal function was within normal parameters.

The additional laboratory tests (Table 1) were intended to narrow the differential diagnosis of anemia in our patient. According to a peripheral blood smear, the mature erythrocyte size differed slightly and some areas had weak staining. Target erythrocytes, teardrop-shaped red blood cells and oval-shaped red blood cells could be seen (Figure 2). Bone marrow aspiration was compatible with hyperplastic anemia, and morphologic signs of megaloblastic anemia (Figure 3). The reticulocyte count was 1.58%, indicating active erythroid proliferation. Therefore, malignant hematological disorders such as myelodysplastic syndromes, leukemia, lymphoma and aplastic anemia could be excluded. An elevated ferritin eliminated iron deficiency or occult bleeding, while a high lactate dehydrogenase and hyperbilirubinemia suggested hemolysis. However, a negative direct Coombs test in our patient could rule out autoimmune hemolytic anemia (AIHA). Furthermore, the presence of target erythrocytes in the peripheral blood, high levels of lactate dehydrogenase and indirect bilirubin, and morphological changes in bone marrow were the potential clues pointing towards thalassemia. Indeed, hemoglobin electrophoresis showed hemoglobin A_2 and F were 6.00% [normal range 2.5–3.2] and 7.80% [normal range

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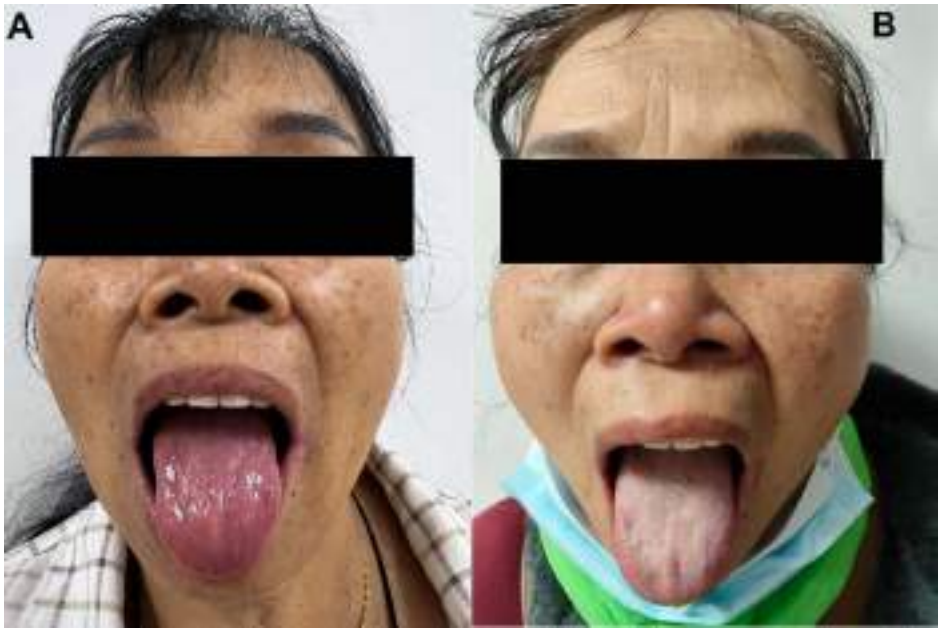

Figure 1 (**A**) An image of a 65-year-old Chinese woman with glossitis (smooth and red tongue dorsum without lingual papillae) caused by pernicious anemia. (**B**) Her tongue appears normal three month after starting treatment with methylcobalamin.

0–0.5]. Gene sequencing showed that the codon 41/42 (-CTTT) mutation in this patient caused β-thalassemia. Most notably, vitamin B_{12} level was 104.9 pg/mL, significantly below the lower limit of detection [normal range 197–771], but the folate (18.26 ng/mL) concentration was normal. Homocysteine level was significantly elevated (165.3 umol/L [normal range 4.0–15.4]). Serum anti-gastric parietal cell antibody (PCA) and anti-intrinsic factor antibody (IFA) were positive. Thyroid-stimulating hormone level was normal. Thyroglobulin antibody and antithyroid peroxidase antibody were negative. A gastroscopy revealed showed chronic atrophic gastritis (Figure 4). Infection with Helicobacter pylori was excluded. Taken together, we diagnosed the patient with both pernicious anemia due to vitamin B_{12} deficiency caused by autoimmune gastritis, and thalassemia.

Table 1 Laboratory Data

Variable	On Evaluation, Outpatient Clinic	Day Since Admission					Reference Range, Adults
		Day 1	Day 3*	Day 7	Day 14	Day 90	
Hemoglobin, g/L	78	76	73	76	87	113	115–150
Hematocrit, %	24.2	23	22.2	24.1	27.9	36.9	35–45
White-cell count, × 10^9/L	3.52	3.09	3.47	4.10	6.17	5.16	3.5–9.5
Differential count, %							
Neutrophils	56.8	53.4	43.5	57.3	63.2	54.5	40–75
Lymphocytes	38.4	42.1	46.7	31	26.9	33.9	20–50
Monocytes	2.8	2.9	5.2	9.3	8.1	8.9	3–10
Eosinophils	1.7	1.3	4.0	2.2	1.3	2.3	0.4–8
Basophils	0.3	0.3	0.6	0.2	0.5	0.4	0–1
Platelet count, ×10^9/L	361	276	351	307	436	310	125–350
Red-cell count, ×10^{12}/L	2.83	2.68	2.56	2.74	3.39	5.31	3.5–9.5
MCV, fl	85.5	85.8	86.7	88.0	82.3	69.5	82–100
MCH, pg	27.6	28.4	28.5	27.7	25.7	21.3	27–34
MCHC, g/L	322	330	329	315	312	306	316–354

(*Continued*)

Table 1 (Continued).

Variable	On Evaluation, Outpatient Clinic	Day Since Admission					Reference Range, Adults
		Day 1	Day 3*	Day 7	Day 14	Day 90	
Reticulocyte count, × 10^{12}/L		0.0423	0.0538	0.1978	0.1678	0.1136	0.024–0.084
Reticulocyte percentage, %		1.58	2.1	7.22	4.95	2.14	0.5–1.5
Red-cell distribution width, %	18.8	19.2	19.2	19.4	17.7	14.4	11.6–14.6
Bilirubin, umol/L							
Total	27.8	38.1	20.2				0–21
Direct	10.8	13.3	8.4				0–8
Alanine aminotransferase, U/L	9						7–40
Aspartate aminotransferase, U/L	17						13–35
Alkaline phosphatase, U/L	41						50–135
Protein, g/L							
Total	65.9						65–85
Albumin	43.6						40–55
Globulin	22.3						20–40
High-sensitivity troponin, ng/mL	0.032						0–0.014
Urea nitrogen, mmol/L	2.71						3.1–8.8
Creatinine, umol/L	45.6						41–81
Creatine kinase, U/L	45.6						41–81
Thyrotropin, uIU/mL		2.57					0.27–4.2
Sodium, mmol/L		139.5					137–147
Potassium, mmol/L		4.07					3.5–5.3
Chloride, mmol/L		101.9					99–110
Glucose, mmol/L		4.87					3.89–6.11
Calcium, mmol/L		2.27					2.11–2.52
Lactate dehydrogenase, U/L		342					120–250
INR		1.07					0.8–1.5
PT, s		12.4					9.8–13.5
APTT, s		27.2					22.5–34
Fibrinogen, g/L		3.65					1.8–3.5
D-dimer, ug/mL		0.23					0–0.5
Ferritin, ng/mL		289.3				175.7	13–150
Homocysteine, umol/L		165.3				9.6	4.0–15.4
Vitamin B_{12}, pg/mL		104.9				384.7	197–771
Folate, ng/mL		18.26				>20.00	3.89–26.8

Note: *First dose of intramuscular vitamin B_{12} given on day 3 of hospital admission.
Abbreviations: MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time.

We prescribed intramuscular vitamin B_{12} (500 μg daily) for our patient's pernicious anemia beginning on the second day of admission, and oral folic acid supplementation. According to several studies, there has been no difference in outcomes between orally and parenterally administered vitamin B_{12}.[1,2] Therefore, switching to orally administered vitamin B_{12} (1.5 mg/day) at discharge is reasonable. Although the patient's blood work abnormalities did not improve significantly during her 7-day hospitalization, her symptoms of painful tongue and reduced sense of taste were resolved. Three months after discharge, at a follow-up visit, her tongue regained its normal appearance (Figure 1B), and her vitamin B_{12} level were normal. However, substitution therapy must be continued indefinitely.

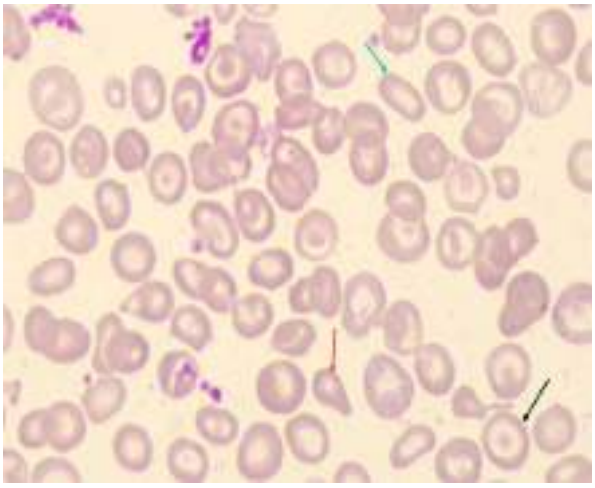

Figure 2 Peripheral blood smear examination showed poikilocytosis: with target erythrocytes, teardrop-shaped red blood cells and oval-shaped red blood cells. Green arrow indicate oval-shaped red blood cell; red arrow indicate teardrop-shaped red blood cell; black arrow indicate target erythrocyte.

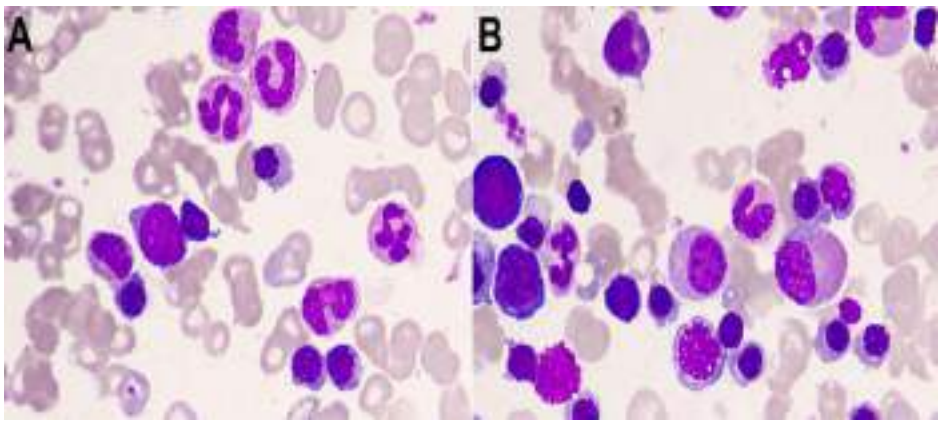

Figure 3 (**A** and **B**) Smears of bone marrow aspirates demonstrating megaloblastic changes: nuclear-cytoplasmic dyssynchrony and nuclear irregularity in erythroid lineage cells, and also nuclear-cytoplasmic dyssynchrony, hypersegmentation, and giant metamyelocytes.

Discussion

We report a case of a patient with pernicious anemia presenting as glossitis in which the diagnosis was delayed because of concomitant thalassemia. Pernicious anemia is a megaloblastic anemia (MA) and mean corpuscular volume (MCV) is the most important parameter in routine blood test data for diagnosing MA. It is easy to diagnose MA when a high MCV occurs in a patient with MA alone. However, MA combined with thalassemia can be masked by the loss of macrocytosis. The prevalence of thalassemia in China is 4–8%, and MA combined with thalassemia is even rarer and easily missed.[3] Pernicious anemia also presents with hemolysis, which is due to the breakdown of abnormal and fragile RBC precursors leads to intramedullary hemolysis and resulting abnormalities in hemolysis tests. However, it still needs to be differentiated from AIHA and requires a relatively extensive work-up. In our patient, AIHA was ruled out due to a negative direct Coombs test, but in rare cases, AIHA can coexist with PA.[4] With continued vitamin B_{12} supplementation, her improvement further suggests that concurrent AIHA was unlikely since true AIHA usually requires glucocorticosteroids, immunosuppressive agents or splenectomy for adequate response.

Autoimmune gastritis (AIG), as a non-self-limiting chronic inflammatory disorder, leads to the destruction of parietal cells in the stomach, resulting in reduced acid output and loss of intrinsic factor, that results in malabsorption of vitamin B_{12} and iron, which cause pernicious anemia and iron deficiency anaemia.[5] In AIG, micronutrient malabsorption causes

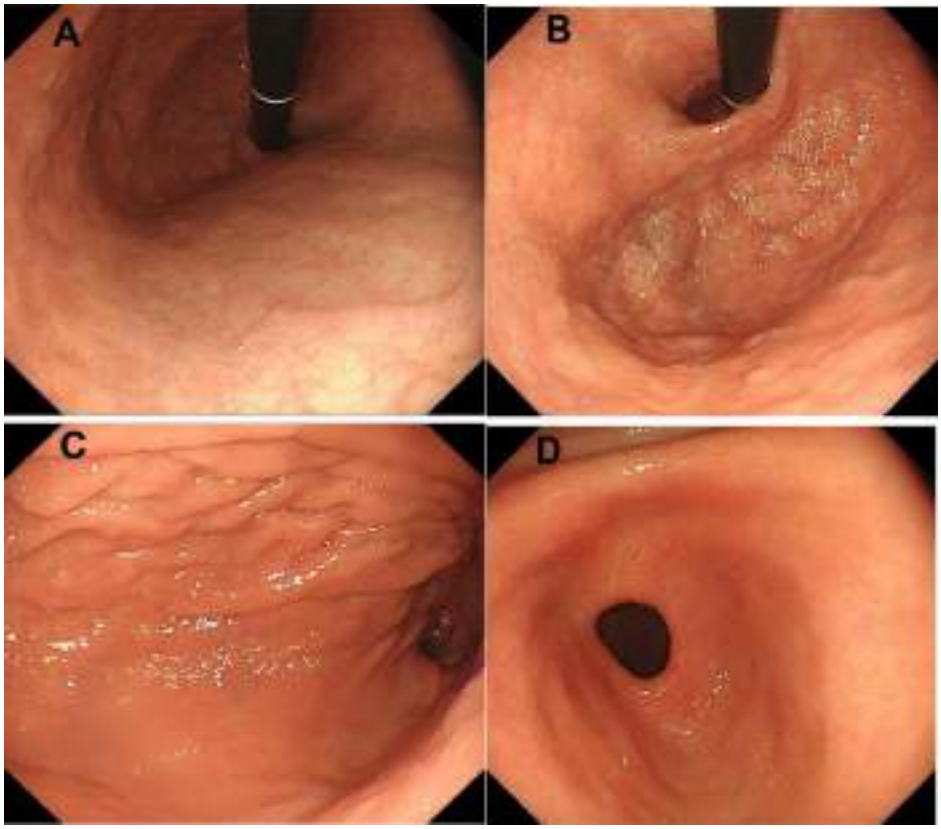

Figure 4 Endoscopic images of the stomach. (**A**–**C**) The typical endoscopic features of atrophic gastritis are apparent, including loss of rugal folds and pallorous-appearing gastric mucosa; due to the thinned gastric mucosa, there is the increased prominence of submucosal blood vessels in the gastric body. (**D**) There is no atrophy of the mucosa of the gastric antrum.

a wide range of clinical manifestations, causing substantial diagnostic delays, resulting in life-threatening and irreversible complications. However, it has been widely accepted for several years that pernicious anemia is a synonym for AIG, which leads to confusion between the two, since pernicious anemia is only one aspect of AIG's clinical picture. Other clinical manifestations such as gastrointestinal symptoms (eg, early satiety, postprandial fullness, nausea, and weight loss) are also very common in patients with AIG. In addition, severe vitamin B_{12} deficiency in patients with AIG may result in neurological damage, causing symptoms such as impaired peripheral nerve function and sensory, abnormal proprioception, ataxia, paresthesia, numbness, mood disorders, cognitive impairment and psychosis. Hyperhomocysteinaemia, infertility, and recurrent miscarriages are other infrequent symptoms of AIG.[5] Despite the fact that patients with AIG can experience unspecific or subtle upper gastrointestinal symptoms, mostly commonly dyspepsia, the majority are asymptomatic before anemia or neurological symptoms develop.

Vitamin B_{12} is essential for various bodily functions, including DNA synthesis, nervous system regulation, amino acid metabolism, and red blood cell maturation in the bone marrow.[6] The most common cause of severe vitamin B_{12} deficiency is autoimmune gastritis, which manifests as pernicious anemia, as seen in our patient. Additionally, vitamin B_{12} deficiency can also occur as a result of other malabsorptive conditions (such as inflammatory bowel disease, or pancreatic insufficiency), after certain procedures (such as bariatric surgery, or ileal resection), restricted diets (such as veganism), or the use of certain medications (such as metformin or proton-pump inhibitors).

Upper gastrointestinal endoscopy and biopsy of the gastric body and antrum are necessary for diagnosing autoimmune gastritis. Clinicians should also consider testing for anti-parietal cell antibodies and anti-intrinsic factor antibodies in patients with histology compatible with autoimmune gastritis. The prevalence of PCAs is estimated at 85–90% among adults with pernicious anemia and AIG, while IFAs are found in 35–60% of these patients.[7,8] Many case series, however, diagnosed AIG solely based on serological markers such as IFAs or PCAs, pepsinogen levels or gastrin-

17, without confirmatory biopsy.[9] In addition, patients with iron or vitamin B_{12} deficiency caused by AIG may not show symptoms, and anemia is usually treated without further investigation into its cause.[10]

Currently, there is no cure for AIG, and immunosuppressive therapy for AIG patients does not currently have long-term trials. Supplementation with micronutrients is the mainstay of treatment for AIG patients. Vitamin B_{12} supplementation is the treatment of choice, regardless of the cause of vitamin B_{12} deficiency. Hematological alterations can be reversed with treatment. It is recommended that newly diagnosed vitamin B_{12} deficiency patients, particularly in patients with critically low levels of serum vitamin B_{12} or with neurological deficits, receive parenteral supplementation to improve their condition rapidly.[5] In theory, oral supplementation with vitamin B_{12} and parenteral administration can both be equally effective for maintenance. According to a Cochrane review comparing oral and intramuscular vitamin B_{12} treatments, both treatments are comparable in the ability to normalize serum vitamin B_{12} levels.[2] There is, however, the possibility that oral supplementation might hinder adherence to treatment because it requires strict monitoring of the patient's treatment and high-dose daily intake, compared to a monthly injection or fewer administering.

Conclusion

In clinical practice, the clinical manifestations of autoimmune gastritis are often not apparent, and even when they are, especially in the early stages, delays in diagnosis are common. Therefore, clinicians should be aware of best practices in diagnosis and management to reduce the possibility of adverse outcomes.

Consent to Publish Statement

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. Consent to publication has been obtained: the Medical Ethics Committee of Shenzhen Longgang Central Hospital approved the publication of the case.

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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.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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CASE REPORT

Encephalitis with Antibodies Against Glial Fibrillary Acidic Protein (GFAP) After Allogeneic Hematopoietic Stem Cell Transplantation: A Rare Case Report and Literature Review

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Abstract: In this report, the patient was a 57-year-old woman who had been diagnosed with aplastic anemia for 3 years. This patient underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). Twenty-four months after allo-HSCT, the patient experienced cognitive dysfunction, memory loss, and involuntary movements. Various central nervous system (CNS) complications may occur after allo-HSCT, which can lead to severe clinical problems. Diagnosis is often difficult because of the absence of distinctive clinical symptoms. In addition, different neurological disorders may show similar symptoms. Although antibodies in the CSF or serum have become well recognized in several CNS disorders, cases of autoimmune CNS disorders after allo-HSCT have rarely been reported. Here, we report the case of a patient who developed encephalitis associated with antibodies against glial fibrillary acidic protein (GFAP) after allo-HSCT. To the best of our knowledge, this is the first report of the involvement of antibodies against GFAP in post-transplantation encephalitis. Of course, all processes met the ethical and patient consents were obtained.

Keywords: glial fibrillary acidic protein, autoimmune encephalitis, allo-HSCT

Introduction

For various hematopoietic diseases, hematopoietic stem cell transplantation (HSCT) is a well-recognized treatment choice.[1] After HSCT, infection, CNS complications, therapy-induced cytotoxicity, autoimmune diseases and graft versus-host disease (GVHD) are common complications.[2–8] Calcineurin inhibitors -related neurological complications account for about 25–59%, and the neurotoxicity caused can be transient isolated symptoms or serious complications, such as Posterior reversible encephalopathy syndrome(PRES), thrombotic microangiopathy(TMA) etc. Post-transplantation complications are mainly related to hematopoietic and immune system hypoplasia. Because of the poor prognosis of CNS complications, early diagnosis and treatment are crucial to achieve a good prognosis.[2] Encephalitis with antibodies against GFAP after allo-HSCT is extremely rare.[9] Hence, its pathophysiology and treatment have not been elucidated. Here, we report the case of a patient who developed encephalitis associated with antibodies against GFAP after allo-HSCT.

Case Report

A 57-year-old woman was diagnosed with aplastic anemia (AA). She failed to respond to Cyclosporin A (CsA) combined with Stanozolol. She was then admitted to our institution to undergo allo-HSCT. The patient received an allogeneic bone marrow transplant from her daughter. The conditioning regimen consisted of antithymocyte globulin (ATG) 2.5 mg/kg on Days −12 to −9, busulfan 0.8 mg/kg on Days −7 to −6, fludarabine 30 mg/kg on Days −5 to −1, and cyclophosphamide 40 mg/m^2 on Days −5 to −4. Methotrexate (15 mg/m^2 on Day +1; 10 mg/m^2 on Days 3, 5 and 11) was administered, and

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Table 1 Anti-GFAP Antibodies Were Detected in the CSF at a Ratio of 1:320 by TBA and Were Positive by CBA

Testing Items	Methods	Result	Reference Value
GFAP	CBA	Positive 1:320	Negative
TBA detection of brain tissue slices	TBA	Positive	Negative

Abbreviations: GFAP, glial fibrillary acidic protein; TBA, tissue-based assays; CBA, cell-based assays; CSF, cerebrospinal fluid.

tacrolimus was administered (1 mg/day) from Day 6 for graft-versus-host disease (GVHD) prophylaxis. Oral acyclovir (0.4 g/day) was administered for herpes simplex virus (HSV) prophylaxis starting on Day 1.

More than two years following transplantation, the patient developed a skin rash without fever, and she developed short-term memory dysfunction and emotional lability. Furthermore, she exhibited involuntary movements of her hands.

In the indirect immunofluorescence detection of the patient's sample in brain tissue slices, there were fluorescence signals in the cerebellum and hippocampus regions. And the pattern was consistent with positive anti-GFAP antibodies. Anti-GFAP antibodies were detected in her cerebrospinal fluid (CSF) at a ratio of 1:320 by tissue-based assays (TBAs) and were positive by cell-based assays (CBAs) (Table 1). Protein levels were normal, and no viruses (HHV-6, cytomegalovirus, Epstein–Barr virus [EBV], herpes simplex virus [HSV], or varicella zoster virus) were detected in the CSF. No obvious abnormality was found in the electroencephalogram of the patient.

Brain magnetic resonance imaging (MRI) showed bilateral hyperintensity on T2-weighted and FLAIR images (Figure 1). Finally, we diagnosed her as having encephalitis with anti-GFAP antibodies. She received methylprednisolone in combination with FK-506, as it was unclear whether her encephalitis was due to GVHD or autoimmune encephalitis.

Discussion

The patient's main manifestations were cognitive and brain function impairment, short-term memory dysfunction, and drowsiness.

According to the literature reports, the reasons for central nervous system (CNS) complications include infection, immunological factors, transplant type, disease type, GVHD, CsA use, stem cell source and conditioning regimen.[4–11]

In this study, we detected anti-GFAP antibodies in the patient's CSF. Anti-GFAP antibodies is essential for diagnosis.[12–16] However, there are also literature reports that GFAP is not a pathogenic antibody. The literature provides evidence for the possible mechanism of anti-GFAP antibody-associated encephalitis, including cytokines, viral infection, autoimmune factors, and GVHD. Concentrations of GFAP and IL-6 in CSF showed a good correlation during the onset

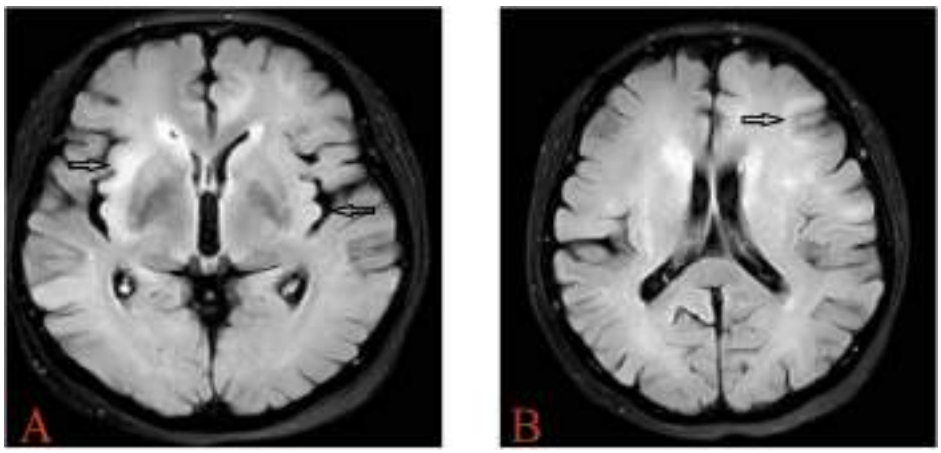

Figure 1 (**A**) MRI on FLAIR image shows well-demarcated bilateral hyperintensity (arrow) in insular. (**B**) MRI shows hyperintensity on T2-weighted and FLAIR images-(arrow) in the limbic system.

of Neuromyelitis optica spectrum disorder.[17] Reduced immune tolerance may promote the emergence of autoimmune diseases while waiting for immune reconstitution after transplantation.[8] While waiting, autoimmune encephalitis with elevated anti-GFAP antibodies in the cerebrospinal fluid may have developed.

GFAP astrocytopathy is an autoimmune disease of the nervous system. Autoantibodies have been detected in the cerebrospinal fluid (CSF) and serum of GFAP astrocytopathy patients. Both tissue-based assays (TBAs) and cell-based assays (CBAs) are recommended methods to detect GFAP antibodies.[16] Both methods confirmed GFAP antibody positivity in the patient whose case is reported here.

We completed cerebrospinal fluid and imaging examinations as well as electroencephalography. Infectious factors should be excluded, but the pathogenicity of autoimmunity or GVHD is still unclear. Brain tissue biopsy or invasive tissue examination may provide more evidence on pathogenicity. The etiology cannot be further demonstrated because of the limitations of the examination. Imaging examinations do a favor in differential diagnosis. Magnetic resonance (MRI) can display various lesions of central nervous system after HSCT.[4] Nevertheless, we chose methylprednisolone in combination with FK-506, and the patient achieved a good response. The patient's cognitive function was restored. Methylprednisolone has been gradually reduced and has been stopped for more than a month. The patient has shown no signs of recurrence.

This may be the first case of anti-GFAP antibody-associated autoimmune encephalitis after allo-HSCT. Therefore, the accumulation of similar cases is necessary to elucidate the pathogenicity and disease specificity and to develop a treatment plan and prognosis.

Patient's Consent for Publication

The current case report was published with informed consent of the patient, whose anonymity was preserved.

Compliance with Ethical Standards

The patient provided written informed consent for publication of the case report. Institutional approval was not required to publish the case details. The data that support the findings of this study are available on request from the corresponding author, Meng Hu, upon reasonable request.

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Disclosure

The authors declare no competing interests in this work.

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REVIEW

A Systematic Review of the Epidemiology and Disease Burden of Congenital and Immune-Mediated Thrombotic Thrombocytopenic Purpura

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Abstract: Congenital (cTTP) and immune-mediated (iTTP) thrombotic thrombocytopenic purpura are serious and rare clotting disorders resulting from a deficiency in the ADAMTS13 enzyme. A systematic review was conducted using the Ovid® MEDLINE & Embase databases to synthesize the epidemiology and burden of cTTP and iTTP worldwide (from January 1, 2010, to February 6, 2020, with an update that covered the period January 1, 2020–February 11, 2022). Outcomes of interest were incidence and prevalence of TTP, incidence of acute episodes, mortality, burden of illness (eg complications, healthcare utilization, patient-reported outcomes) and disease management. A total of 221 eligible observational studies were included. The incidence rate of acute episodes ranged from 0.19–0.35 person-years in adult patients with cTTP, and 1.81–3.93 per million persons per year for iTTP in the general population. Triggers of acute episodes were similar for cTTP and iTTP, with pregnancy and infection the most commonly observed. Exacerbation in patients with iTTP varied widely, ranging from 2.4–63.1%. All-cause mortality was observed in 0–13.4% of patients with cTTP, across studies and follow-up periods, and in 1.1% (median follow-up: 0.4 years) to 18.8% (1 year) of patients with iTTP during acute episodes. Cardiovascular, renal, and neurological disease were common complications. TTP also led to work disturbances, feelings of anxiety and depression, and general activity impairment. TTP treatment regimens used were generally reflective of current treatment guidelines. The evidence identified describes a high patient burden, highlighting the need for effective treatment regimens leading to improvements in outcomes. Considerable evidence gaps exist, particularly for disease epidemiology, patient-reported outcomes, costs of disease management, and associated healthcare resource utilization. This review may help increase disease awareness and highlights the need for additional real-world studies, particularly in geographical regions outside the United States and Western Europe.

Keywords: thrombotic thrombocytopenic purpura, epidemiology, burden of illness, disease management, patient-reported outcomes, ADAMTS13

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy (TMA), primarily caused by a deficiency in the von Willebrand factor (VWF)-cleaving enzyme ADAMTS13 (A disintegrin and metalloproteinase with thrombospondin motifs 13).[1–3] This deficiency can be the result of mutations in the *ADAMTS13* gene (hereditary/congenital TTP; cTTP) or, more commonly, result from ADAMTS13 autoantibodies (acquired/immune TTP; iTTP).[3] An ADAMTS13 activity of <10% is required to confirm the diagnosis of TTP,[2,4–6] and the distinction between cTTP and iTTP relies on genetic analysis and/or an anti-ADAMTS13 autoantibody assay.[2]

The annual incidence of TTP is estimated to range between 2–6[4] and 3–11 cases per million persons.[7] Recent research suggests that the diagnostic criteria for TTP should consider patients with microangiopathic hemolytic anemia and thrombocytopenia (MAHAT), without neurologic/renal abnormalities and fever (previously part of the "classic pentad" of diagnostic symptoms).[2,3,5] Thus, estimated ranges for the annual incidence of acute TTP episodes are not adequately summarized in the literature to date. Acute episodes are considered a true medical emergency and are associated with a mortality rate of >90% if left untreated.[2,8,9] Damage to major organs may also result in transient ischemic attack, stroke,

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myocardial infarction, or acute kidney injury.[1,10,11] Triggers for acute episodes include infections, pregnancy, autoimmune disease, blood/marrow transplant and exposure to certain medications.[3,12,13]

The current standard of care for managing acute TTP episodes is to restore ADAMTS13 levels using plasma therapy, either by plasma infusion for cTTP or plasma exchange (PEX) for iTTP.[3,11,14,15] Immunosuppressive or anti-CD20 therapy (eg rituximab) and anti-VWF therapy (caplacizumab) are also options for iTTP,[3,15] while prophylaxis with regular plasma infusions has been used to prevent acute episodes in cTTP. However, TTP treatment guidelines highlight a lack of high-quality evidence supporting long-term outcomes with currently available therapy.[15]

Due to the rarity of TTP, the body of evidence to describe the natural history of the disease and to identify unmet needs for TTP is limited but growing. Therefore, a systematic literature review was conducted to analyze data on the epidemiology and burden of TTP worldwide in order to better inform the management of TTP and clinical decision-making. This review covers the epidemiology, patient characteristics, natural history, burden of illness, mortality, and real-world treatment of TTP.

Materials and Methods

Scope of the Review

A systematic review was conducted which covered the period from January 1, 2010, to February 6, 2020 (date of last search) (PROSPERO CRD42020172273) using methods developed by the Cochrane group and the National Academy of Medicine, formerly the Institute of Medicine of the National Academy.[16,17] An update was conducted covering the period January 1, 2020, to February 11, 2022 (date of last search).

The systematic review was based on the PICOTS (Population, Intervention or Exposure, Comparator, Outcomes, Time Period, Setting) criteria. The target population included adult and pediatric patients with iTTP or cTTP. Intervention was the local/regional standard of care prescribed in the real-world setting and the comparator could be the local/regional standard of care (or no comparator). Outcomes of interest in the general population were incidence and prevalence of the disease; incidence of acute episodes; all-cause and TTP-related mortality; patient characteristics (age and sex distribution, race and ethnicity, comorbidities at baseline); natural history of disease (ADAMTS13 activity pre-treatment, during treatment and post-treatment, disease course, disease triggers, disease-related complications, organ damage biomarkers); burden of illness (hospital length of stay [LOS], healthcare costs, patient-reported outcomes/quality of life [PROs/QoL]); and disease management. The study setting was observational/non-interventional.

Search Strategy and Information Sources

Literature searches using the Ovid® MEDLINE & Embase databases 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): Population of interest (iTTP and cTTP, or unspecified TTP when distinction between subtypes was not available) and parameters of interest (incidence, prevalence, mortality, relapse, age distribution, sex ratio, disease management, natural history, organ damage, complications, and burden of illness). Search strategies are detailed in Supplementary Table 1. In addition, pragmatic searches were performed using Google and the Google Scholar search engines, as well as websites of learned or clinical societies and related conference proceedings (annual meetings) and relevant patient organizations (Supplementary Table 2) to identify publications not indexed in MEDLINE and Embase. The reference lists of retained publications were screened for additional relevant sources (referred to as "snowballing").

Eligibility Criteria

The inclusion criteria consisted of observational studies (eg cohort studies, cross-sectional studies, non-comparative cohort studies [case series]) published in English that included patients with iTTP or cTTP (or unspecified TTP when distinction between subtypes was not available) either as the study population or as a sub-group analysis. Other criteria included studies that reported on outcomes of interest as defined in the PICOTS, original research articles published as full-text or conference proceedings (ie, posters, abstracts), and reviews (systematic, non-systematic, and meta-analyses

[for snowballing only]). Case reports, editorials, letters to editors, opinions, clinical trials (phase I–III), nonclinical studies, experimental studies, and studies describing preliminary results later reported as full text were excluded.

Selection of Studies

Duplicate sources were removed using automated procedures. During the first stage of the selection process, titles and/or abstracts were screened using the predefined eligibility criteria by two independent reviewers, with conflicts resolved by a third assessor. During the second stage, eligibility was confirmed by in-depth review of full texts and reasons for exclusion at this 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 independently by two reviewers in a standardized data extraction form, with conflicts resolved by consensus or by a third assessor for completeness. Data items extracted included general study information (source, citation, publication type, geographical coverage); study methods (study period, study design, data collection method, data source); target population (population of interest [unspecified TTP, cTTP, iTTP, mixed] and targeted age); and study population (study inclusion/exclusion criteria, diagnostic criteria, number of TTP patients). The following study outcomes were also extracted: study follow-up, patient characteristics (age at first symptoms/diagnosis, sex distribution, race and ethnicity, comorbidities, biomarkers at baseline or during follow-up), and estimates of outcomes of interest.

Results of the systematic review were synthesized qualitatively and there was no pooling of estimates through a meta-analysis. A range of estimates was provided when possible, and outlying estimates were qualitatively assessed. Data for all outcomes were reported separately according to TTP type (ie, unspecified TTP, cTTP, iTTP). Risk of bias was not assessed due to the rarity of the disease.

Studies reporting outcomes have been placed into separate categories based on the type of data source used to identify TTP patients, namely registry-based studies, healthcare database studies (claims-based or integrated healthcare studies; from here on referred to as database studies) and other studies including multicenter, single-center, and survey-based studies. The separation of database studies from registry and other clinical studies was necessary due to the difference in diagnosis/identification of patients between these study types. As there is no unique International Classification of Diseases (ICD) code for TTP, research databases that use claims coding algorithms to identify cases of TTP are not based on confirmed diagnoses,[18] unlike clinical studies.

Results

Search Results

Following literature searches and the removal of duplicate sources, 207 literature sources were eligible and included in the review. Pragmatic searches and snowballing yielded 14 additional relevant sources. Thus, a total of 221 references were included in the review (Figure 1; Supplementary Figure 1). The majority of studies reported on unspecified TTP (n=113) or on iTTP only (n=88), while the remaining studies reported on cTTP only (n=15) or both cTTP and iTTP (n=5). Key characteristics of the studies included in this manuscript are presented in Supplementary Table 3.

Incidence and Prevalence of TTP

Incidence

There was substantial heterogeneity in incidence estimates for unspecified TTP (n=4 studies) and iTTP (n=7 studies), with a single study reporting the incidence for cTTP (Figure 2A). The reported incidence for unspecified TTP was 8.92 per million person-years between 1996 and 2012 based on the US Oklahoma TTP-Hemolytic Uremic Syndrome [HUS] registry study,[19] 3.88 cases per million new admissions between 2003 and 2013 in the UK Hospital Episode Statistics [HES] database,[20] 0.8 cases per million between 2011 and 2014 in a multicenter study in the UK,[21] and 1.91 per million per year between 2012 and 2019 in a single-center study in Canada.[22] The incidence for cTTP from 2005 to 2013 was 0.3 cases per million based on medical charts from a single-center study in Israel.[23] For iTTP, incidence ranged between 0.77 and 2.67 per million persons

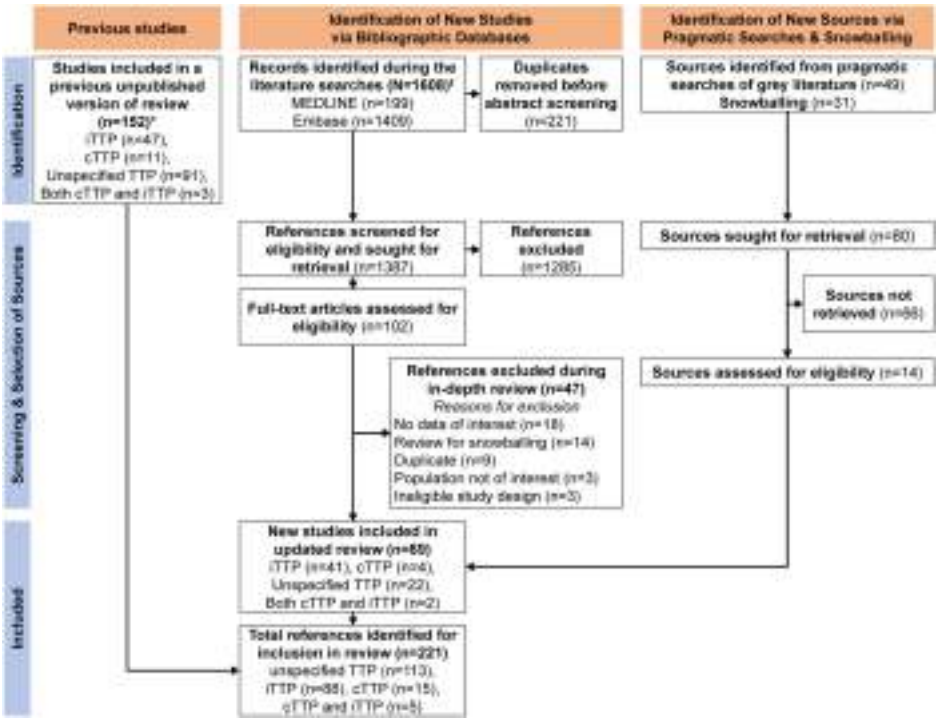

Figure 1 PRISMA flow chart for the selection of sources.
Notes: *Search period January 1, 2010–January 31, 2020 (See Supplementary Figure 1); †Search period January 1, 2020–February 11, 2022.

per year in European multicenter studies (assessment period: 2014–2016 in Germany, 1998–2007 in central Norway, and 2015–2017 in Spain).[24–27] The incidence of iTTP reported in an Israeli single-center study from 2005 to 2013 was similar to that observed in Europe (2.0 per million per year).[23] In Japan, the incidence of iTTP reported in 2018 was 0.4 per million persons according to a Nara Medical University Registry study.[28]

Prevalence

A summary of the prevalence of TTP is presented in Figure 2B. Three studies reported on the prevalence of unspecified TTP:[29–31] 13 adult-onset cases per million in a French TMA registry study,[29] 9.9 per million adults aged 19–64 years in the US (estimated from 2439 eligible patients among 245 million patients screened; IBM MarketScan® Research Database),[30] and 1–2.7 per million in the US (HealthCore Integrated Research Database™).[31] The prevalence of cTTP varied widely across three studies, with a global prevalence of 0.4–16.7 per million persons based on the Orphanet disease information resource,[32] 0.86 cases per million from the French TMA registry,[33] while a Norwegian multicenter cohort study investigating a hypothesis that central Norway may have higher cTTP prevalence than elsewhere reported 3.1 diagnosed or suspected cases per million based on the population for the whole of Norway and an outlier of 16.7 diagnosed or suspected cases per million based on the population of central Norway.[26] The authors hypothesized that the higher cTTP prevalence in central Norway may be associated with a high frequency of the *ADAMTS13 c.4143_4144dupA* mutation, thought to have arisen in that small area.[26] Six studies reported prevalence for iTTP, with estimates of 3.43 per million persons in the US (Optum-Humedica Database),[34] 12.99 per million persons in France (Orphanet disease information resource),[32] 19 per million persons in the US (Oklahoma TTP-HUS registry),[35] and 19–21.44 per million per year in Spain (multicenter study).[24,25] In the French TMA registry, one person with childhood-onset iTTP was estimated per million children.[36] Additional details for studies reporting the incidence and prevalence of TTP are available in Supplementary Table 4.

Patient and Disease Characteristics

Patient characteristics of individuals with cTTP and iTTP are summarized in Table 1.

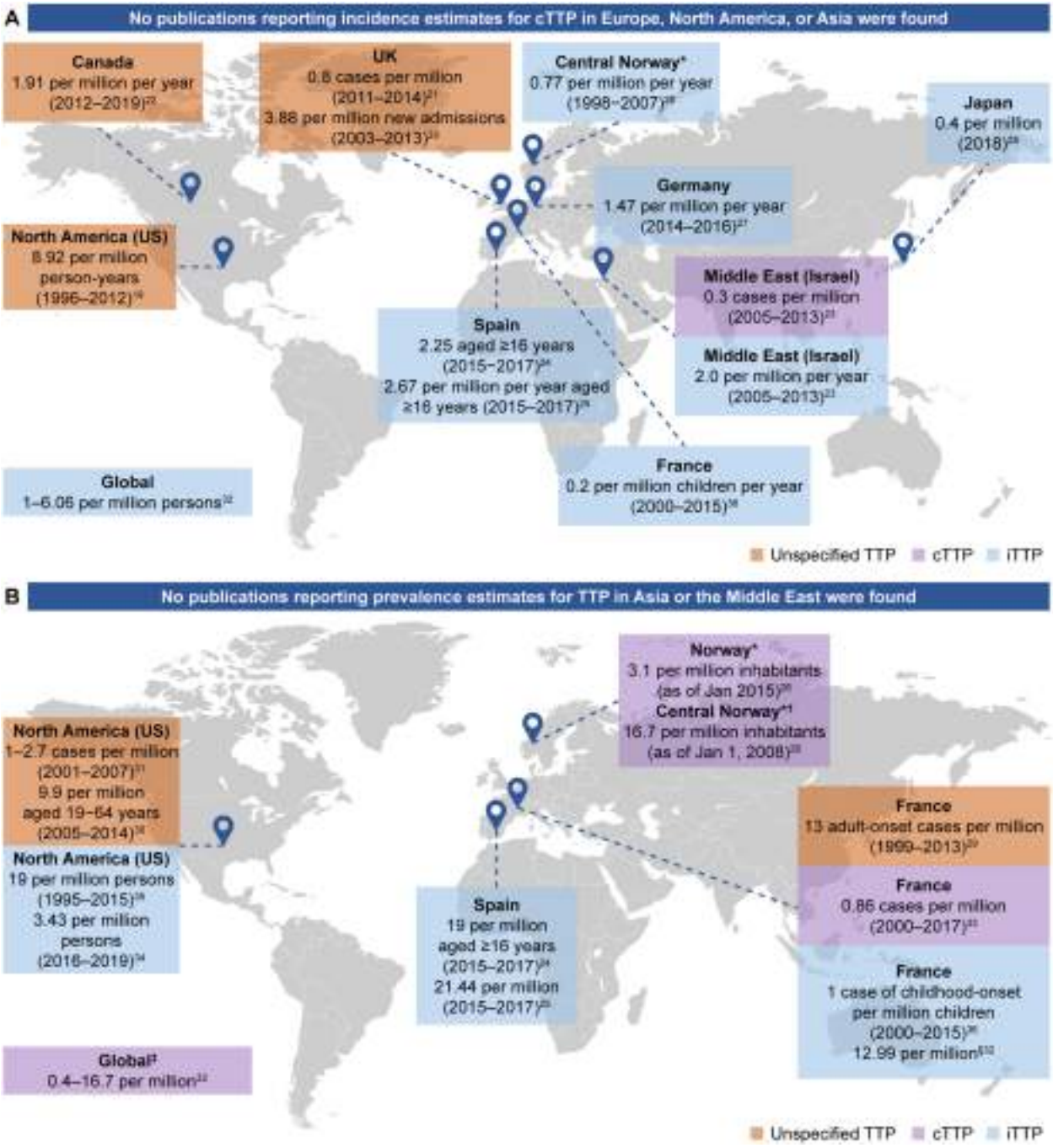

Figure 2 The incidence (**A**) and prevalence (**B**) of unspecified TTP, cTTP and iTTP.
Notes: *Considered both diagnosed or suspected cTTP cases; †Based on a study of central Norway with specific *ADAMTS13* mutations. ‡Values derived from reported data: 1 per 60,000 to 2,500,000. §Value derived from reported data: 1 per 77,000.
Abbreviations: cTTP, congenital thrombotic thrombocytopenic purpura; iTTP, immune thrombotic thrombocytopenic purpura; TTP, thrombotic thrombocytopenic purpura.

Age at Symptoms Onset and Age at Diagnosis

The onset of cTTP symptoms typically occurred between birth and 35 years of age.[8,37–40] Age at cTTP diagnosis followed a similar trend. Based on International hTTP and UK TTP registry studies, cTTP was typically diagnosed between early childhood (3.5 years) and late twenties.[8,11,37,50]

Table 1 Patient Demographic and Clinical Characteristics

Category	cTTP	iTTP
Age at symptom onset	**Registry studies** • Median: 4.5–4.6 years[8,37] • 74.3% experienced any TTP-related symptom(s) at birth; 37.1% experienced a first acute episode <1 year of age[38] • Ranged from neonatal period to 35 years[39] **Multicenter study**[40] • 53.3% during the neonatal period (<28 days) • 23.3% during the prepubescent phase (<14 years) • 6.7% at age ≥14 years	**Registry studies** • Median: 41–54 years[41–45] **Single-center studies** • Median in pediatric patients: 14 years[46] • Median: 41–49 years[47,48] • Mean: 45.7 years[49]
Age at diagnosis	**Registry studies** • Median: 16.7–18.7 years[8,37,50] • Two peaks in presentation (median): early childhood (3.5 years) and during pregnancy in women (29 years)[11] **Single-center study** • Median: 27 years[23] **Patient-interview study** • Mean: 27.5 years in the US[51]	**Registry studies** • 40–46 years (median)[52–56] • Mean 39.4–40.7[57] • Pediatric patients – 13 years (median)[36,38] **Database studies** • Median: 64 years[58] • Mean: 48.8–53.1[59,60] **Single-center and multicenter studies** • Median: 33–47 years[23,56,61–65] • Mean: 43.7–47.0 years[66–70]
Frequent comorbidities (≥10%)	**Registry studies** Cardiovascular • Arterial thrombotic diseases[a]: 28.0%–36.0%[8,37] Hepatic • Hyperbilirubinemia in neonatal period: 25.0%–43.0%[8,37] • Jaundice: 49.0%[8] Neurological • Epileptic seizure, headache: 22.0%[37] Renal • Renal insufficiency: 25.0%–31.0%[8,37]	**Registry studies** Autoimmune disease • Autoimmune disease (type unspecified): 14.7%–41.9%[41,43,54,71] • Autoimmune thyroiditis[b]: 10.8%–32.0%[41,54] • Lupus: 7.0%–13.0%[41,54,56,72–74] Cardiovascular • Hypertension: 26.9%–60.2%[52,56,72–74] • Obesity: 29.2%[73] • Dyslipidemia: 17.5%[73] • Hyperlipidemia: 32.6%[72] • Atrial fibrillation: 10.5%[72] Renal • CKD: 16.8%–28.2%[56,72,73] Other • Diabetes: 15.4%–27.1%[52,56,72–74] **Database studies** Psychiatric • Depression: 16.7%[59] Renal • Renal disease: 18.0%[75] Other • Diabetes: 12.5%–25.7%[59,75] • Cancer: 11.8%–18.3%[59,75] • Chronic pulmonary disease: 13.0%[75] • Rheumatologic disease: 10.6%[75] • Stroke/TIA: 10.4%[59] **Single-center and multicenter studies** Autoimmune disease • Autoimmune disease (type unspecified): 12.1%–24.3%[63,64] • Systemic lupus erythematosus: 38.2%[76] • Connective tissue disease: 10.0%–14.6%[47,76] Cardiovascular • Hypertension: 17.5%[64] Psychiatric • Psychiatric disease: 11.0%[13] Other • Flu-like syndrome: 16.2%[64]

Notes: [a]Stroke, transient ischemic attack, myocardial infarction and other (not specified); [b]Including Hashimoto's thyroiditis.
Abbreviations: cTTP, congenital thrombotic thrombocytopenic purpura; iTTP, immune thrombotic thrombocytopenic purpura; US, United States.

Compared with cTTP, the onset of symptoms in patients with iTTP occurred later in life, at a median age ranging between 41 (interquartile range [IQR]: 35–48) and 54 years (IQR: 37–65).[41–44,47,48] Diagnosis of iTTP was typically confirmed at the time of symptom onset. The median age at diagnosis ranged between 33 (range: 12–64) and 64 years (IQR: 47–74).[23,52–56,58,61–65]

Sex Distribution

There was generally a lower proportion of males than females in both adult and pediatric cTTP and iTTP populations. The proportion of males among patients with cTTP ranged from 7% to 55.6%, even though cTTP is an autosomal recessive disorder and therefore should have the same risk regardless of sex.[11,23,37–39,75,77–80] Two UK TTP registry

studies reported that the proportion of males varied according to age at the onset of cTTP symptoms,[11,77] with a higher proportion observed with early-onset disease (68%) compared with late-onset (7–25%). Similar to cTTP, the proportion of male patients with iTTP typically ranged from 17.0% to 50.0% across studies.[12,23,41–48,54–56,58–63,65–69,72–76,81–106] Out-of-range estimates were found in two studies, one including patients who provided a self-reported diagnosis in a UK-based survey (2.9% male patients)[107] and one Italian multicenter study in which patients presented with an initial TTP episode (64.9% male patients).[64]

Race and Ethnicity

Two studies using the international hTTP registry reported that White/Caucasian race was the most common among patients with cTTP (53.0–86.2%).[8,37] For patients with iTTP in registry studies across Europe and the US (TTP, TMA, iTTP/aHUS, and US state registries), the proportion of White/Caucasian patients ranged between 21.9% and 98.0%.[41,43,53,54,56,71–74,81,86–89] For other study types, the proportion of White/Caucasian patients reported was 61.7%[59] in the US Medicare Fee-for-Service database study, and 39.0–97.1% in single-center and multicenter studies across the US and Italy.[48,64,93,99–101,108] In Europe, White/Caucasian iTTP patients were predominant (67.1% to 98.0%).[41,43,48,54,64,71,81,86] Nine US-based studies reported African Americans as the predominant racial group (50.5% to 78.0%),[46,53,56,72–74,88,89,102] while six US-based studies reported that iTTP patients were predominantly White/Caucasian (54.0% to 78.9%).[59,87,93,100,101,108]

Comorbidities

In studies using the hTTP registry, nearly half of patients with cTTP had a history of jaundice,[8,37] while approximately 30.0% of patients had arterial thrombotic diseases and/or renal insufficiency,[8,37] and 22.0% had neurological disorders[37] (Table 1). Comorbidities observed in ≥10% of patients with iTTP included autoimmune disease (12.1–41.9%[41,43,54,63,64,71]), hypertension (17.5–60.2%[52,56,64,72–74]), obesity (29.2%[73]), diabetes (12.5–27.1%[52,56,59,72–75]), chronic kidney disease (16.8–28.2%[56,72,73]), psychiatric disease (11.0–16.7%[13,59]), cancer (11.8–18.3%),[59,75] and stroke/transient ischemic attack (10.4%[59]). Estimates were consistent across registry studies [n=9],[41,43,52,54,56,71–74] database studies [n=2],[59,75] and single-center and multicenter studies [n=4].[47,63,64,76]

Incidence of Acute Episodes and Triggers

Incidence of Acute Episodes

A limited number of studies reported incidence of acute episodes in unspecified TTP (n=1),[109] cTTP (n=4),[8,37,110,111] and iTTP (n=4)[25,27,34,35] (Supplementary Table 5). Definitions of acute episodes were either missing[110,111] or varied between studies due to different data sources and methods.[8,25,27,34,35,37,109,110] The reported incidence of acute TTP episodes was 14.9 per 100,000 adult hospitalizations per year according to a US National Inpatient Sample database study.[109] Incidence rates of acute episodes/person-years in the International hTTP registry study were 0.19–0.35 in adult cTTP patients[37,110] and 0.77 for cTTP patients aged ≤18 years.[111] Between 2006 and 2017, the median number of episodes per year was 0.10 per patient (range: 0.02–8.91).[8] The incidence of acute episodes in cTTP was higher in females compared with males, in patients aged <10 years than in those aged ≥10 years, and in those who did not receive plasma prophylaxis[37,111] (Supplementary Table 6). For iTTP, the reported annual incidence of acute episodes was 1.81 per million persons in the US Optum-Humedica database,[34] 2.10 per million persons in a German multicenter study,[27] 3.10 per million persons in the Oklahoma TTP-HUS registry,[35] and 3.93 per million persons in individuals aged >16 years from a Spanish multicenter nationwide survey.[25]

Triggers of TTP Episodes

Triggers of acute episodes were similar for cTTP and iTTP, with pregnancy and infection being the most commonly observed.[8,11,12,35,37,42,50,64,69,103] In patients with iTTP, surgery[12,64,103] and medication/drug use (including antidepressants, anti-inflammatories, oral contraceptives, anti-epileptics, clopidogrel, vaccination, and recreational drugs [cocaine])[12,13,64,69] were other reported triggers of acute episodes.

Incidence of Relapsed Episodes, Relapse Rate, Exacerbation, and Refractory Disease

Incidence of Relapsed Episodes/Relapse Rate

For cTTP, 55.6% of patients experienced a relapse during follow-up (unknown period) in a study using the International and Milan HUS TTP registry[39] and 83.3% of patients (with recurrent episodes leading to diagnosis) relapsed between 1998 and 2007 in a Norwegian multicenter study.[26] During a mean follow-up of 2.8 years (IQR: 1.6–4.9 years), 0.4 episodes/year (IQR: 0.1–1.05) were recorded in the International hTTP registry between 2012 and 2016.[110]

For iTTP, the incidence of relapse ranged widely (9.4% to 48.6%) across studies that used a similar definition.[34,36,41,42,55,65,82,84,89,90,112–114] Estimates did not vary according to the type of data source (registry, administrative claims database, medical chart review). Heterogeneity across studies can be explained through methodological differences (ie, sample size, follow-up period, definitions of relapse, eligibility criteria) and clinical management of iTTP during acute episodes and during follow-up (type of treatment and treatment setting [on-demand, prophylaxis], etc).[64,67,81,82,86,103,115] For instance, relapse was observed in 1.1% of iTTP patients treated with a triplet regimen in France (PEX, immunosuppression with corticosteroids and rituximab, and caplacizumab; French TMA registry study),[81] and 3.5% of patients receiving caplacizumab plus other approved treatment per physicians' decision in the UK (UK TTP registry).[86] In another single-center study in China, the low incidence of relapse (5.3%) was due to the short assessment period (1 month post-discharge).[103] In a meta-analysis of relapse in patients receiving rituximab or conventional treatment (primarily of PEX and corticosteroids), the incidence of relapse in patients receiving only rituximab as an acute or preemptive treatment was 15.8% and 13.9%, respectively, while 58.5% of patients who did not receive preemptive treatment relapsed.[115]

The cumulative incidence of relapse was found to increase over time.[67] At 24, 48, 72, and 120 months post-treatment initiation, the cumulative incidence was lower in patients treated with either rituximab or cyclophosphamide in addition to PEX and steroids (n=28) compared with those treated only with PEX and steroids (n=10), according to a single-center study in the US (2010–2019).[67]

Incidence of Refractory TTP Disease

Data on refractory cTTP were lacking in the literature. Specifically, two TMA registry studies reported very different estimates among iTTP patients of 1.1% (French TMA registry)[81] and 30.1% (US Thrombotic Microangiopathy [USTMA] registry).[89] The lower incidence of refractory iTTP may be due to patients receiving aggressive therapy with combination treatment (intensive frontline triplet regimen of therapeutic PEX, immunosuppression with corticosteroids and rituximab, and caplacizumab).[81] Refractory disease was reported in 12.5–17.9% of patients included in single-center and multicenter studies.[12,25,63]

Incidence of Exacerbation

No data were found on the incidence of exacerbation in patients with cTTP. For patients with iTTP, incidence of exacerbation varied widely, ranging from 2.4% to 63.1%.[12,25,34,36,59,63,64,74,81,86,94,102,116,117] Incidence of exacerbation appeared to depend on treatment, with lower estimates observed in patients receiving a combination of PEX, corticosteroids, rituximab, and caplacizumab (2.4%[86] and 3.3%[81]). According to study type, estimates ranged between 24.7% and 46.7% in two studies based on the French TMA registry and the Alabama registry,[36,74] while a lower range of 12.5% to 17.2%[34,59] was reported in database studies. A wider range of 13.6% to 63.1% was observed across single-center and multicenter studies.[12,25,63,94,102,116,117]

Disease Biomarkers – Change in ADAMTS13 Levels or Activity

No data were found on change in disease biomarkers in cTTP patients. However, a number of registry and single-center and multicenter studies reported on ADAMTS13 levels or activity in patients with iTTP, at diagnosis/first acute episode, during remission, and at relapse.[36,73,82,84–86,94,116,118]

In patients presenting with ADAMTS13 activity <10% at diagnosis or relapse in an Italian hematology department, all had normal ADAMTS13 activity (>50%) after treatment with PEX.[94] Similarly, in a study of the UK TTP registry study, ADAMTS13 levels increased considerably after PEX and caplacizumab treatment in patients after a confirmed

diagnosis of acute TTP.[86] In patients who survived ≥30 days after preemptive rituximab, ADAMTS13 activity was detectable in >80% of patients at Day 30 and at 3 months post-treatment.[82] However, at 6 months, ADAMTS13 was undetectable in most patients. In patients with child- and adolescent-onset iTTP who survived their initial episode, all had detectable ADAMTS13 activity (>40% of normal) at remission.[36]

A strong association between the decline in ADAMTS13 activity and the occurrence of relapse was found in patients with iTTP.[84,116] An Italian multicenter study found that a combination of anti-ADAMTS13 antibodies levels ≥20 U/L and ADAMTS13 activity <20% strongly predicted relapse during remission (P=0.0004).[116] In the Prospective Observational Registry for iTTP in Germany, 44.9% of patients had a persisting normal ADAMTS13 activity (≥50%) during remission; 55.1% of patients had ADAMTS13 activity of <50% at least once, and 18.6% of those had continuous activity of <10% without relapse.[84] Of patients who relapsed, 77.8% had an ADAMTS13 activity of <2% before relapse. In another study based on the same registry, 70% of patients in remission had normal ADAMTS13 activity (≥50%) at enrollment, with 4% of patients experiencing a rapid decline from >80% to <1% at 3 months after study enrollment, and 2% experiencing a slow decline from >100% to 4.6% at 4.5 months after study enrollment.[85] In patients with a history of iTTP investigated for ADAMTS13 activity every 3 months,[118] 10.3% experienced persistent severe ADAMTS13 deficiency in remission and 10.3% experienced subsequent severe ADAMTS13 deficiency after a median follow-up of 17 months.

Disease Complications

Overall, in patients with iTTP or cTTP, frequently reported complications (affecting ≥10% of patients) included cardiovascular, neurological, and renal disease (Table 2). Disease complications for cTTP were reported in two studies using the UK TTP registry. Short-term disease complications during acute episodes included stroke and transient ischemic attack, observed in 24.7% of patients,[11] while persistent cognitive symptoms (a long-term complication) were observed in 33.3% of patients at a median follow-up of 33 months.[119] These results were consistent with findings from a review of published case reports in which major morbidities consisted of stroke/transient ischemic attack (38.9%), end-stage kidney disease (25.9%), and neurological abnormalities (11.1%) at a median follow-up of 10 years in patients who survived an initial episode[120] (Supplementary Table 7). Other observed complications include neonatal hyperbilirubinemia (43%),[37] arterial thrombotic disease (36%),[37] renal insufficiency (31%),[37] ≥1 arterial thromboembolic event (transient ischemic attack, stroke, myocardial infarction; 25.3%),[50] neurological disorders (22%),[37] and miscarriage/stillbirth (11.0%).[11]

For iTTP, frequently reported short-term disease complications include acute kidney injury (91%),[68] neurological events (36%[68] and 47.4%[62]), stroke or transient ischemic attack (39.5%),[62] altered mental state (15.8%),[62] and seizure (15.8%).[62] Several long-term complications were observed 2 to 8 years after iTTP diagnosis, including persistent neurological impairment (26.1–60.0%),[46,48,119] major cardiovascular events (28.6%),[72] hypertension after surviving initial iTTP episode (24.2%),[52] and stroke (13.1–18.2%).[72,73]

Mortality

For cTTP, the rate of all-cause mortality in registry-based studies included no deaths in a pediatric study (N=35)[3] and ranged from 4.8% (during 371 person-years of prospective follow-up; N=87) to 6.8% (median 8.3 year follow-up; N=73) in other populations with a defined follow-up period (no age definition).[11,37,110] Causes of death consisted of stroke (80.0%) and cancer unrelated to TTP (20.0%) in one UK TTP registry study,[11] and sudden death due to unknown cause (55.6%), cerebral infarction (11.1%), sepsis (11.1%), uremia (11.1%), and suicide (11.1%) in a Japanese cTTP registry survey-based study.[78]

Overall, the rate of all-cause mortality during or following acute episodes among patients with iTTP ranged from 1.1% (median follow-up: 0.4 years) to 18.8% (1 year).[12,35,42,45,63,64,73,74,81,82,90,122] Three US and French database studies reported 30-day mortality between 7.4% and 21.0% after index hospitalization with standard treatment,[59,71,91] while two US retrospective multicenter studies reported 90-day mortality between 5.5% and 7.3% (with all of the 7.3% of deaths actually occurring within the 30 days after presentation).[93,108] Mortality differences between studies should be interpreted with caution due to differences in populations and study designs. In a meta-analysis reporting all-cause mortality among patients with acute episodes (initial or

Table 2 Short- and Long-Term Complications

Reference (Study Type, Study Period)	Patients (N) Duration of Follow-up	Disease-Related Complications
cTTP		
Short-term complications (at presentation or following diagnosis)		
Alwan, 2019[11] (Registry, 2003–2018)	73 Median: 8.3 years (range: 0.9–40.2)	Cardiovascular • Stroke: 19% (n=14) • Transient ischemic attack: 8% (n=6)
Long-term complications (Range 2.75–10 years)		
Alwan, 2020[119] (Registry, 2005–2019)	12 Median: 33 months (range: 2–130)[a]	• Persistent cognitive symptoms[b]: 33.3% (n=4) • Neurocognitive compromise (in those tested): 75% (n=3)
Borogovac, 2022[120] (Literature review, 2001–2020)	226 Median: 10 years (range: 1–47)[c]	Initial major morbidity among 217 patients: 33.6% (n=73) Subsequent major morbidity in 54 patients who survived initial comorbidity: 68.5% (n=37)
Unknown assessment time		
Sakai, 2021[78] (Registry, Apr–Sep 2020)	55 NR	Any persistent organ damage: • FFP prophylaxis: 39.0% (n=16) • FFP on-demand: 0%
Tarasco, 2021[37] (Registry, 2018–2019)	87 Median: 4.2 years (range: 0.01–15)	Possible cTTP-related comorbidities at enrollment[d]: Cardiovascular • Arterial thrombotic disease: 36% (n=30) • Venous thrombotic disease: 3.6% (n=3) Hepatic • Neonatal hyperbilirubinemia: 43% (n=36) Neurological • Neurologic disorders: 22% (n=18) Renal • Renal insufficiency: 31% (n=26)
Alwan, 2019[11] (Registry, 2003–2018)	73 Median: 8.3 years (range: 0.9–40.2)	Medically significant complications: Cardiovascular • Stroke/transient ischemic attack: 24.7% (n=18) • Pulmonary hemorrhage: 1.4% (n=1) Other • Miscarriage/stillbirth: 11.0% (n=8) • Third-trimester pregnancy complications excluding miscarriage: 5.5% (n=4) • Visual defects: 4.1% (n=3) • Facial palsy: 2.7% (n=2) • Retinal vein thrombosis: 1.4% (n=1) • Seizures: 1.4% (n=1)
Mansouri, 2015[50] (Registry, 2015)[e]	83 NR	• ≥1 arterial thromboembolic event (transient ischemic attack, stroke, myocardial infarction): 25.3% (n=21) • Venous thromboembolism: 1.2% (n=1)
Rurali, 2015[39] (Registry, 1996–2013)	18 NR	At last follow-up: • Chronic kidney disease: 27.8% (n=5)
iTTP		
Short-term complications (defined per publication)		

(*Continued*)

Table 2 (Continued).

Reference (Study Type, Study Period)	Patients (N) Duration of Follow-up	Disease-Related Complications
Pollissard, 2021[59] (Database, 2010–2018)	2279 (aged ≥18 years) 20.7–31.7 months	During index hospital stay: Cardiovascular • Stroke or myocardial infarction: 9.9% (n=226)
Pascual-Izquierdo, 2021[25] (Multicenter cross-sectional study, 2015–2017)	329 NR	After hospital admission for first episodes or relapsed episodes of iTTP (exact timing not specified): Cardiovascular • Arterial thrombosis: 1.0% (n=2) • Venous thrombosis: 1.0% (n=2) • High blood pressure: 0.5% (n=1) Other • Plasma allergy: 18.1% (n=35) • Thrombosis of central venous catheter: 4.6% (n=9)
Renaud, 2021[62] (Single-center cohort study, 2005–2020)	38 NR	At presentation with first iTTP episode: Neurological • Neurological event within one week after hospital evaluation: 47.4% (n=18) • Altered mental status: 15.8% (n=6) • Neurological sequelae at discharge: 5.3% (n=2) Cardiovascular • Stroke or transient ischemic attack: 39.5% (n=15) Other • Seizure: 15.8% (n=6)
Ramachandran, 2020[68] (Single-center cohort study, 2014–2019)	10 NR	At hospital presentation: • Neurological complications: 36% • Acute kidney injury: 91%
Huang, 2021[69] (Single-center cohort study, 2013–2017)	55 NR	During the hospital stay: • Mean sequential organ failure assessment score was significantly higher in non-survivors (mean score: 12.1 [SD: 3.3]) than in survivors (7.7 [2.1])
Long-term complications (median follow-up 33 months to 8 years where reported)		
Joly, 2016[36] (Registry, 1999–2017)	41 (child-onset and adolescent-onset iTTP) Median 8 (range 1–16) years	In patients who survived their first iTTP episode: Renal • Impaired renal function: 4.9% (n=2) Other • Hemiparesis: 4.9% (n=2) • Deafness: 4.9% (n=2) • Blindness: 4.9% (n=2)
Mancini, 2020[42] (Registry, 2002–2018)	153 Median 4.9 (95% CI: 3.7–6.1) years	In patients followed after their first iTTP episode: • Cancer: 2.0% (n=3)
Alwan, 2020[119] (Registry, 2005–2019)	119 Median 33 (range 2–130) months	iTTP patients presenting with neurological symptoms: • Underwent neuropsychology assessment (due to self-reported persistent cognitive symptoms): 26.1% (n=31)
Upreti, 2019[73] (Registry, 1995–2018)	170 Median (IQR): 3.08 (0.66–7.79) years	In patients treated with PEX: Cardiovascular • Stroke unrelated to an acute iTTP episode (ie, occurring during remission after recovery from iTTP): 2.57 per 100 patient-years (prevalence was 13.1% [n=18]) • Median time from first iTTP diagnosis to stroke (IQR) was 2.8 (0.8–10.0) years

(*Continued*)

Table 2 (Continued).

Reference (Study Type, Study Period)	Patients (N) Duration of Follow-up	Disease-Related Complications
Han, 2015[121] (Registry, 1995–2013)	52 NR	Patients who recovered from TTP and underwent cognitive impairment over 11 years[f]: • Results for immediate (p=0.0124) and delayed memory (p=0.0228) in 2014 were significantly worse vs results from 2006 (n=15) • Other cognitive components such as attention, language, and visuospatial components did not significantly differ between 2006 and 2014
Little, 2017[52] (Registry, 1995–2015)	78 Median 6.4 years	• Chronic kidney disease: 6.4% (n=4; of which, 40% had mild CKD) ○ Of these patients, other conditions included: pre-existing hypertension (n=2), diabetes (n=1), diabetes and hypertension (n=1) • After surviving an initial TTP episode (n=66), 24.2% (n=16) developed hypertension
Brodsky, 2021[72] (Registry, 1995–2020)	181 Median 7.6 years	During clinical remission: • Major cardiovascular event: 28.6% (n=43) ○ Stroke: 18.2% (n=33) ○ Non-fatal myocardial infarction: 6.6% (n=12) ○ Cardiac revascularization: 4.9% (n=9) ○ Fatal myocardial infarction: 0.6% (n=1)
Pollissard, 2021[59] (Database, 2010–2018)	2279 Mean 25.1 months	Incidence rates of post-discharge complications (per 100 person-years): • Cardiovascular: 14.4 ○ Hypertension: 10.0 ○ Heart failure: 7.4 ○ Cerebrovascular events: 5.3 ○ Stroke or transient ischemic attack: 3.4 ○ Deep vein thrombosis: 1.2 ○ Myocardial infarction: 1.2 • Metabolic conditions and renal impairment: 12.8 ○ Diabetes: 5.3 ○ Renal disease: 7.2 • Neurological ○ Cognitive and physical impairment: 8.3 ○ Mental health conditions: 8.1 ○ Schizophrenia: 4.8 ○ Depression: 4.0 ○ Anxiety disorder/post-traumatic stress disorder: 3.9 ○ Dementia: 3.6 ○ Seizures/epilepsy: 1.5 • Other ○ Fatigue: 4.7 ○ Fever: 4.6 ○ Dizziness/accidents/falls (emergency admissions for contusions, breaks, hematomas): 4.2 ○ Focal deficits: 2.3 ○ Pain or discomfort: 1.1 ○ Pulmonary embolism: 0.8 ○ Urticaria: 0.7 ○ Coma: 0.6 ○ Anemia: 11.3
Tiscia, 2021[64] (Multicenter cohort study, 2013–2021)	74 Median 60 months	Patients with first iTTP episode: • Ischemic stroke (3 years after episode): 1.1% (n=1) • Autoimmune disease[g]: 3.4% (n=4)

(*Continued*)

Table 2 (Continued).

Reference (Study Type, Study Period)	Patients (N) Duration of Follow-up	Disease-Related Complications
Pascual-Izquierdo, 2021[25] (Multicenter cross-sectional survey, 2015–2017)	193 (aged ≥16 years) NR	After resolution of acute episodes: Cardiovascular • Cardiological complications: 0.5% (n=1) Neurological • Central neurological complications: 2.1% (n=4) • Psychiatric complications: 2.1% (n=4) Renal • Renal complications: 0.5% (n=1) Other • Avascular hip necrosis: 1.0% (n=2) • Peripheral polyneuropathy: 0.5% (n=1) • Hepatitis E virus infection: 0.5% (n=1)
Riva, 2020[48] (Single-center cross-sectional study, 2015–2016)	35 Median 36 months	After last acute episode, in remission phase: • Persisting subjective neurological impairment: 48.6% (n=17) • Significantly poorer scores according to neuropsychological tests were observed in iTTP patients vs the general population: ○ Direct memory (mean difference: −5.87 [95% CI: −8.57, −3.17]) ○ Deferred memory (mean difference: −1.67 [95% CI: −2.32, −1.02]) ○ Focused attention (mean difference: −10.63 [95% CI: −15.81, −5.44]) ○ Sustained and divided attention (mean difference: 65.09 [95% CI: 47.23, 82.94]) • No differences in neuropsychological assessments were found between patients with ADAMTS13 levels <45% compared with those with levels ≥45% during remission
Graciaa, 2020[46] (Single-center cohort study, 2001–2009)	15 (aged ≤19 years) NR	Patients presenting at hospital with iTTP: • Persistent or worse neurologic complaints 6 to 8 months following disease onset: 60% (n=9)

Notes: [a]Time from acute TTP episode to neuropsychology assessment; [b]Evaluated using the following cognitive domains: premorbid optimal level of functioning (National Adult Reading Test), current general intellectual functioning (Wechsler Adult Intelligence Scale [WAIS]-III Verbal or Performance Scale IQ), verbal and non-verbal memory, naming, perception, frontal executive function and speed of information processing; [c]Follow-up of surviving patients with major morbidities (n=54); [d]Including potential cTTP-related complications resulting from previous episodes. [e]Year of publication; [f]Test (Repeatable Battery for Assessment of Neuropsychological Status [RBANS]); [g]Sjogren syndrome, undifferentiated connective tissue disease and autoimmune hypothyroidism.

Abbreviations: CI, confidence interval; CKD, chronic kidney disease cTTP, congenital thrombotic thrombocytopenic purpura; FFP, fresh frozen plasma; IQR, interquartile range; iTTP, immune thrombotic thrombocytopenic purpura; NR, not reported; PEX, plasma exchange; TTP, thrombotic thrombocytopenic purpura.

relapse), pooled, unweighted mortality was 3.0% and 10.7% of patients treated with rituximab or conventional treatment, respectively.[115] Mortality data from studies with no reported follow-up are presented in Supplementary Table 8.

Reported TTP-related mortality ranged from 0.9% to 13.3%, regardless of study type and type of episode (initial or acute).[12,13,45,54,63,64,86,90,112,114,122–124] Frequently reported causes of death included relapse/exacerbation of TTP (1.5–77.8%);[56,71,73,106,122] cardiovascular events (1.4–27.6%);[56,73,116] infection (10.5–13.8%);[56,69] and TTP refractory to PEX (2.6–88.9%).[62,74,106,125] Variation in study type and type of episode may have contributed to the wide estimate ranges for cause of death.

Disease Burden

Patient-Reported Outcomes

cTTP had an extensive negative impact on all areas of quality of life, including daily activities such as the ability to work and/or study, mental health (specifically feelings of anxiety and depression), financial distress, and mood swings according to a single-center study conducted in the US.[51] Patients also had low confidence, experienced anger and frustration, and felt burdened by treatment. Similar to cTTP, patients with iTTP reported issues with daily life, including

difficulties with work or school activities[36,48,107] as well as with family or social interactions.[36] Patients with iTTP also suffer from feelings of anxiety,[48,107] depression,[48,107,121] and cognitive impairment.[101,107]

Costs

A limited number of studies report on costs associated with TTP. In the US, the total mean costs associated with TTP were estimated at USD 236,278 (standard deviation [SD]: 8439) in survivors and USD 784,606 (SD: 151,799) in non-survivors, based on the Kids' Inpatient Database and the National Inpatient Sample study between 2003 and 2014.[126] According to the IBM MarketScan® research database, between 2005 and 2014, the total median costs per TTP patient admission were USD 42,593 (IQR: 18,904–110,424).[30] Based on a single-center study from China (2009–2018), inpatient median costs were higher in patients treated with PEX (24,965, range: 3305–137,685) than in those treated with plasmapheresis (22,829, range: 4197–57,185), although the currency was not reported.[127]

No data on costs associated with cTTP were found. However, among patients with iTTP in Japan, median total cost per patient was USD 40,897 (IQR: 24,204–64,012) based on the Japanese Diagnosis Procedure Combination inpatient database between 2010 and 2017.[58] In a study of the US Medicare Fee-for-Service database and Inovalon MORE2 registry database (2010–2018), mean costs associated with index hospitalization (based on ICD codes for thrombotic microangiopathy and therapeutic plasma exchange) were USD 15,587.50 (SD: 13,227.75) and median costs after index hospitalization were USD 3243.25 per month over a mean follow-up of 25.1 months.[59]

Healthcare Resource Utilization (HCRU)

Studies reporting hospital LOS for patients with iTTP were identified (Supplementary Box 1). The mean hospital LOS per admission ranged from 12 to 20 days,[13,25,59,68,108] while the median hospital LOS per admission ranged from 12 to 19 days in TTP-HUS, TMA, and TTP registry studies;[35,81,86] 20 to 45 days per admission in the Japanese Diagnosis Procedure Combination inpatient database and French national hospital discharge database;[58,60] and 9 to 28 days per admission across single-center and multicenter studies.[12,24,62,64] Patients remained in the intensive care unit (ICU) for a mean duration of 8.3 days in a study using the US Medicare Fee-For-Service and Inovalon MORE2® databases[59] and 5.5 days in a multicenter study in Spain.[25] The median ICU LOS was 8 days in a French national hospital discharge database[60] and 4 to 7 days in single-center and multicenter studies.[24,62]

Disease Management

The most frequently cited therapies for the management of cTTP were regular plasma prophylaxis and on-demand plasma infusion[8,26,37,38] (Box 1). For iTTP, PEX, corticosteroids, or rituximab were commonly prescribed as on-demand therapy.[25,43,49,62,64,69,76,81,88,90,92,93,105,106]

Box 1 Treatment Patterns of on-Demand and Prophylaxis Therapy

cTTP
Prophylaxis therapy
Registry studies • Regular prophylactic treatment was initiated in 67.1% of 73 patients age ≥16 years[11] • Most patients were treated with regular plasma prophylaxis: 57%,[37] 70.9%[8]   ○ 80.0% in child-onset and adolescent-onset population[38] • FFP was utilized in 41.1%,[11] 55.6%,[39] and 74.5%[78] of patients **Multicenter study** 45.5% were treated with regular plasma prophylaxis[26]

(*Continued*)

Box 1 (Continued).

On-demand therapy

Registry studies
- 29.1% of patients were treated with on-demand plasma therapy (hereditary TTP Registry[8])
- On-demand FFP: 25.5%,[78] 61.3%,[37] and 100%[39]
- On-demand treatment (FFP for age ≥16 years, intermediate purity factor VIII concentrate for age <16 years): 12.3%[11]

Database-based studies
Exchange transfusion during neonatal period: 45.5%[26]

iTTP

Prophylaxis therapy

Registry studies
Products used among patients who received immunosuppressive treatment pre-emptively:[88]
- Corticosteroids: 53.8%
- Rituximab: 30.8%
- Others (Not reported): 15.4%

Multicenter study
Maintenance treatment with rituximab during remission (patients aged ≥16 years):[25]
- Initial episode: 3.1% of 128 episodes

Relapsing episode: 16.9% of 65 episodes

On-demand therapy

Registry studies
First-line treatments used to treat first or relapsing TTP episodes:
- PEX (87.4–100%)[43,81]
  - 92.9% (patients aged ≥12 years)[90]
  - Median number of PEX sessions ranged from 14 (in patients who had the onset of the acute episode at ≥65 years of age) and 11 (patients aged <65)[43]
  - Median duration of PEX treatment: 5 days (range, 4–7) to 7 days[81,86]
- Corticosteroids (82.5–98.8%)[43,81,86]
  - 95.3% (patients aged ≥12 years)[90]

Rituximab treatment usage:
- To treat acute episodes: 20.3%–63.3%[43,83,88,90]
- Commonly used as a second-line treatment in refractory or exacerbated patients[88,90]

Caplacizumab treatment usage:
- Median duration: 33–36 days[81,83]

Other additional treatments included:
- Cyclosporine: 4.7%[90]
- Cyclophosphamide: 1.2%,[86] 2.2%,[81] and 13.0%[90]
- Bortezomib: 0.6%[81] and 5.9%[86]
- Vincristine: 1.7%[81] and 6.5%[90]

Single-center and multicenter studies
- PEX range: 89.1%–100%[49,62,64,69,76,93,105]
  - Median number of PEX sessions ranged from 6 to 15[62,64,69,83,93]
  - Mean duration of PEX treatment: 16.2 days[25]
- Corticosteroids: de novo (97.7%) and relapsing (92.3%)[25]

Rituximab treatment usage:
- To treat acute episodes: 20.2%–48.4%[64,69,93,105,106]
- More frequently administered among relapsing episodes (41.5%) than initial episodes (14.1%) when used as first-line treatment[25]
- Commonly used as a second-line treatment in refractory or exacerbated patients[25,92]

Caplacizumab treatment usage:
- 10.5% of patients with a first acute iTTP episode (2005–2020) treated with caplacizumab[62]
- 2.6% of patients who experienced their first episode (2013–2021) received caplacizumab and/or vincristine[64]
- Median duration (2018–2019): 34 days (range, 2–211)[49]

Other additional treatments included:
- Cyclosporine: 1.5%[25]
- Cyclophosphamide: 1.5% (de novo) and 3.0% (relapse)[25] and 4.0%[93]
- Bortezomib: 1.0%,[93] and 1.5% (de novo and relapse)[25]
- Vincristine: 0.8%[93] and 3.1% (de novo)[25]

Abbreviations: cTTP, congenital thrombotic thrombocytopenic purpura; FFP, fresh frozen plasma; iTTP, immune thrombotic thrombocytopenic purpura; PEX, plasma exchange; TTP, thrombotic thrombocytopenic purpura.

Prophylaxis Treatment

For cTTP, plasma infusion prophylaxis was administered to prevent the occurrence of TTP episodes in 41.1% to 80.0% of patients across all study types.[8,11,26,37–39,78] For the prophylactic treatment of iTTP, 53.8% and 30.8% of patients received corticosteroids or rituximab, respectively, in a USTMA registry study,[88] while rituximab was used for remission maintenance in 3.1% and 16.9% of the initial and relapsing episodes, respectively, in a Spanish multicenter study.[25]

On-Demand Therapy

A greater number of studies reporting data on on-demand treatment for managing acute TTP episodes were identified for iTTP compared with cTTP (Box 1). The proportion of patients with cTTP receiving on-demand therapy with fresh frozen plasma (FFP) ranged from 25.5% to 100% across all study types.[8,26,39,75,78] For patients with iTTP, 87.4% to 100% of patients received on-demand treatment with PEX,[43,49,62,64,69,76,81,93,105] 82.5% to 98.8% received corticosteroids,[25,43,81,86,90] and 20.2% to 63.3% received rituximab.[43,64,69,83,88,90,93,105,106] Other treatments used for iTTP included caplacizumab,[49,62,64,81,83] cyclosporine,[25,90] cyclophosphamide,[25,81,86,90,93] bortezomib,[25,81,86,93] and vincristine,[25,49,64,81,90,93] with a low rate of use (<15%) reported for most of these versus PEX, corticosteroid, or rituximab as on-demand treatments.

Post-Discharge Clinical Management for Patients with iTTP

Data in the literature indicate a lack of standardized post-discharge practices.[12,73,81,84] In registry studies, ADAMTS13 monitoring was conducted weekly during a follow-up period of 127 days after PEX administration (French TMA registry)[81] or every 3 months post-discharge (78.2% of patients had ≥2 visits with median time between visits of 91 days; German TTP registry).[84] In a Johns Hopkins TMA registry study, 30.6% of patients were tested for ADAMTS13 activity at least 3 months following an acute episode (median number of measurements during remission: 3 [range:1–18]).[73] A single-center study reported ADAMTS13 measurement frequency every 3–6 months in 62.5% of episodes and every 12–14 months in 37.5% of episodes, with a median of five measurements per patient.[12]

Discussion

This systematic review provides an overview of the burden of TTP, a rare and serious condition defined by acute and relapsed episodes that is associated with significant mortality if appropriate treatment is not provided in a timely manner. Furthermore, TTP is associated with serious comorbidities in addition to short- and long-term complications, and exerts a substantial negative impact on patient QoL, daily activities, and mental health.

Although there was heterogeneity in reported prevalence estimates, epidemiological evidence highlights the rarity of TTP.[19,21,22,24–28] There were substantial geographical disparities across reports for unspecified TTP, including a large difference in prevalence estimates between France (13 cases per million)[29] and the US (1–2.7 cases per million).[31] The majority of cTTP estimates from Global, French and Norwegian studies, were very low (0.4, 0.86, and 3.1 cases per million, respectively).[26,32,33] However, there was one prevalence estimate for cTTP in central Norway that was a noticeable outlier (16.7 cases per million).[26] This was based on a single study in central Norway, which had a hypothesis that the region may have a higher cTTP prevalence than elsewhere, possibly related to the high reported frequency of the *ADAMTS13 c.4143_4144dupA* mutation, and the inclusion of diagnosed or suspected cTTP cases. In addition, methods for case identification may have influenced the estimates reported for Norway, with a systematic case-finding strategy used for central Norway but not the whole country. The estimated prevalence for the whole country was 3.1 cases per million.[26] iTTP prevalence estimates were also heterogeneous but low (3.43 in the US[34] to 21.44 per million in Spain[25]). Overall, these data suggest that cTTP and iTTP meet the criteria for ultra-rare conditions (prevalence of less than one case per 50,000 individuals).[128]

The International Society on Thrombosis and Haemostasis (ISTH) diagnostic guidelines cite the annual incidence of TTP as 2–6 per million individuals,[4] while unspecified TTP studies identified by this review reported an incidence rate ranging from 0.8 cases per million to 8.92 cases per million person-years.[19–22] Due to finding only one study with cTTP incidence (0.3 cases per million),[23] comparisons with iTTP are limited. The wide range found in population-based estimates may be explained by variations in reported population size, parameters for defining the disease, and the time periods in which the studies were conducted (Supplementary Table 4).

Determining the incidence of such a rare disease can include challenges. For example, in a US study validating administrative claims codes for TTP in the HealthCore Integrated Research Database™, the positive predictive value (PPV) of the initial claims coding algorithm used was 46% (ie, 46% of claims were assessed to have definite evidence to support TTP diagnosis), while the PPV of a refined algorithm was 72%, highlighting the difficulty of accurately confirming diagnoses used in healthcare databases.[18] In our literature review, however, there did not appear to be

a trend for higher or lower incidence or prevalence reported in healthcare database studies compared with registry, single-center, or multicenter studies. Other factors that can affect the incidence and prevalence of TTP include age, and for iTTP, sex, race-ethnicity, obesity, infection and inflammation, in addition to potential genetic risk factors.[129,130] Referral to expert centers may also impact epidemiology data, as diagnosis of rare diseases is often substantially delayed without access to specialists,[131] and patients who live close to tertiary medical facilities are more likely to be accurately diagnosed than those who live further away.[132]

Patient characteristics reported in identified studies may indicate change in practice over time and were inconsistently aligned with known risk factors. Age at diagnosis for cTTP and iTTP typically occurred around symptom onset,[11,23,50,52,54–56,58,61–65] which appears to reflect improvements in the time required to make a clinical diagnosis. For example, during the whole enrollment period, the international hTTP registry reported a median age at cTTP diagnosis that was over 10 years later than the median age at cTTP symptom onset, but for patients diagnosed in recent years, time from symptom onset to confirmation of disease diagnosis had dropped to days or weeks with the use of ADAMTS13 activity assays.[8,37] A female predominance was reported for both cTTP and iTTP,[11,12,23,41–45,47,48,54–56,58,59,61–63,65–69,72–77,79,81–91,93–106] with female sex an established risk factor for iTTP.[3] White race was commonly reported in patients with cTTP[8,11,37] and those with iTTP in European studies.[41,43,48,54,64,71,81,86] Black race is an established risk factor for iTTP,[3,133] with a sevenfold higher incidence reported among individuals who are Black vs non-Black.[134] However, there was no predominance of Black race in patients with iTTP reported in reviewed studies, which may be influenced by differences in access to care or levels of participation in healthcare studies (such as registries).[135–137] US-based studies reported either African American[46,53,73,74,88,89,102] or White/Caucasian[59,87,93,100,101,108] as the most common racial group.

Of utmost importance when considering TTP epidemiology is the incidence of acute TTP episodes, as they are associated with a substantial mortality rate.[4] ISTH guidelines prioritize ADAMTS13 activity testing at acute TTP episodes where there is a high probability of TTP, although others have highlighted the importance of ADAMTS13 testing and treatment with appropriate therapies during remission as well as at acute episodes.[2,138] Although the definition of an acute episode varied due to different data sources and study methods, the incidence of acute episodes was considerable and found to be consistent with relevant clinical presentations, medical events and treatment. Tarasco et al 2021 reported that the incidence of acute episodes varied by age and sex, further suggesting that age may be a driver of acute episodes, with early childhood representing a period of risk for patients with cTTP in particular.[37] In addition, pregnancy has been reported to precipitate TTP, with women presenting with a first acute episode of both cTTP and iTTP during pregnancy in the UK TTP registry.[139]

TTP-related ADAMTS13 deficiency gives rise to acute episodes with typical MAHAT and subacute manifestations,[5,11,110,140] which result in a variety of serious short- and long-term disease complications such as organ damage and mortality.[4,5,110] As cardiovascular, renal, and neurocognitive disorders are common manifestations of TTP,[2] it may be difficult to distinguish comorbidities from disease-related complications. However, their presence contributes to the substantial disease burden for patients with TTP. Although no direct data on the management of patients with TTP with multiple comorbidities/complications were uncovered in this review, it is understood that multimorbidity results in a large economic burden on health systems and society.[141] One of the main challenges of managing TTP is the occurrence of relapsed episodes. Relapses can occur in up to 50% of patients who survive their initial episode, and the timing may be unpredictable, occurring close to achievement of remission or months later.[142] Relapse rates identified in this review ranged between 55.6% and 83.3% for cTTP[26,39] and between 9.4% and 48.6% for iTTP.[34,36,41,42,55,65,82,84,89,90,112–114] Variation in relapse estimates can be explained through methodological heterogeneity regarding study type, sample size, follow-up period for relapse rate assessment, patient profile, and clinical management of acute episodes (type of treatment and treatment setting [on-demand, prophylaxis], etc).[64,65,67,82,86,103,115]

In addition to the unpredictability of relapse, refractory disease and disease exacerbations add a further degree of complexity to disease management. A strong association was found between the decline in ADAMTS13 activity and the occurrence of relapse in iTTP,[84] in keeping with known disease pathophysiology.[10] International treatment guidelines acknowledge that there are practical issues around the cost, resource utilization, and patient commitment necessary for

regular ADAMTS13 monitoring during remission.[15] However, given the association between ADAMTS13 activity and relapse in iTTP,[84] routine ADAMTS13 testing would be a valuable addition to current guidelines.

TTP mortality observed for both cTTP and iTTP in studies with a defined assessment/follow-up period was aligned with the generally accepted rate of between 10% and 20%, even with treatment.[143] Data on all-cause and TTP-related mortality in the current review indicate a substantial mortality burden for patients with cTTP and iTTP, with specific causes of death linked to typical consequences or complications of the disease. It is hoped that regular ADAMTS13 monitoring could help to reduce the risk of relapse and mortality, but more research in this area is needed.

Given the broad range of disease symptoms, comorbidities, and complications, it is unsurprising that studies in this review reported that both cTTP and iTTP negatively affected patient QoL, impacting everyday activities, including the ability to work and/or study[36,48,51,107] and mental health.[48,51,107,121] A limited number of PRO studies were identified despite the negative impact of TTP on QoL, which may be due to the rarity of the disease. Similar to what was found for other outcomes, only one study reported on QoL for cTTP, and a limited number of studies reported on costs associated with unspecified TTP (n=3) and iTTP (n=2), with the majority reporting US-based costs. Consequently, there is a need for further research on PROs and on the economic burden of TTP in different regions. Based on the evidence identified, costs associated with TTP are substantial, with an unspecified TTP admission having a total cost of USD 42,593,[30] for example. Owing to the limited evidence available, the main cost drivers of managing TTP remain unclear.

Different disease management approaches were documented in the literature for cTTP and iTTP, and treatment use generally reflected current ISTH treatment guidelines for TTP, which recommend plasma prophylaxis for cTTP and use of PEX, corticosteroids, or rituximab as on-demand therapy for iTTP.[15] Although caplacizumab represents the first anti-VWF drug to receive a regulatory approval for the treatment of iTTP since 2018, and is now recommended for the treatment of first or relapsing acute iTTP episodes,[15] few studies of this treatment were identified in the systematic review.[49,62,64,81,83] This is likely due to the timing of approval versus studies identified in addition to challenges with the availability of caplacizumab.[15] Other contributing factors may include treatment side effects, cost, requirement of cotreatments to remove the underlying autoantibodies, and the necessary clinician experience with caplacizumab use and monitoring protocols.[15]

A considerable variability in outcomes was reported in this review, likely resulting from differences in study inclusion criteria, research methodologies, study follow-up, and study type. Differences in disease awareness, diagnostic criteria for TTP, definitions of TTP, definitions of acute episodes, and clinical management of TTP across geographic areas and different time periods may also have contributed to the heterogeneity of the data. Some relevant sources may not have been captured in the systematic literature search, as not all studies are published in peer-reviewed journals. To mitigate this limitation, our methodology included pragmatic searches of the gray literature, as well as "snowballing". For most outcomes, a larger body of evidence was identified in the literature for iTTP than cTTP. This is likely to be a consequence of disease epidemiology, with more than 95% of all TTP cases identified as iTTP, while the remainder are classified as cTTP.[4] Specifically for cTTP, an ultra-rare disease, data on epidemiology, disease characteristics, and burden of disease were scarce. For TTP in general, very limited data were identified for regions other than Europe, North America (predominantly the US), and to a lesser extent, Asia. Consequently, data may be considered generalizable only to Europe and the US. The lack of data on aspects of this rare disease indicates a need for additional real-world analyses, particularly outside of Europe and the US.

Conclusion

The evidence identified in this systematic review describes a high burden of illness associated with TTP, including serious acute episodes, mortality, comorbidity, and disease-related complications, in addition to poor QoL. This review also highlights the limited data available on the epidemiology of cTTP and iTTP, PROs, costs of disease management, and associated HCRU. Substantial unmet needs remain, including effective treatment regimens leading to improvements in complications and disease-related mortality. The findings in this review may help increase disease awareness and inform decision-making for disease management and future research studies for the benefit of patients with TTP.

Abbreviations

ADAMTS13, A disintegrin and metalloproteinase with thrombospondin motifs 13; CI, confidence interval; CKD, chronic kidney disease; cTTP, congenital thrombotic thrombocytopenic purpura; FFP, fresh frozen plasma; HCRU, healthcare resource utilization; HES, Hospital Episode Statistics; HUS, Hemolytic Uremic Syndrome; ICD, International Classification of Diseases; ICU, intensive care unit; IQR, interquartile range; ISTH, International Society on Thrombosis and Haemostasis; iTTP, immune-mediated thrombotic thrombocytopenic purpura; LOS, length of stay; MAHA, microangiopathic hemolytic anemia; MeSH, Medical Subject Headings; NR, not reported; PEX, plasma exchange; PICOTS, Population, Intervention or Exposure, Comparator, Outcomes, Time Period, Setting; PPV, positive predictive value; PROs, patient-reported outcomes; QoL, quality of life; SD, standard deviation; TMA, thrombotic microangiopathy; USD, US dollar; USTMA, US Thrombotic Microangiopathy; VWF, von Willebrand factor; TTP, thrombotic thrombocytopenic purpura; UK, United Kingdom; US, United States.

Data Sharing Statement

The datasets, including the template data extraction form and data extracted from the included studies, are available upon request from Ragy Saad (ragy.saad@takeda.com) at the Global Evidence and Outcomes department at Takeda.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, or 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.

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ORIGINAL RESEARCH

Prediction of Viable CD34 Count in Harvested Product by Peripheral Blood Hematopoietic Progenitor Count of Automated Hematology Analyzer Undergoing Hematopoietic Stem Cell Transplantation

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Introduction: The CD34+ hematopoietic cell count was used to define cell harvest goals. Successful peripheral blood stem cell transplantation depends on infusion of an appropriate number of HPCs to achieve rapid and durable hematologic recovery.

Purpose: In this study, we evaluated the use of the Hematopoietic Progenitor Cell count program on the Sysmex XN-3000 hematology analyzer as an effective parameter for enumerating CD34+ cells.

Patients and Methods: Whole blood samples from 144 subjects who are either healthy donors or patients scheduled to undergo peripheral blood stem cell collection were collected and hemopoietic stem cells were quantified using CD34 cell enumeration by flow cytometry and XN-HPC by hematology analyzer.

Results: The correlation between the two methods was high (r = 0.766; 95% CI: 0.702–0.818). Passing–Bablok showed an intercept at 3.45 (2.54 to 4.74) with a slope of 0.78 (95% CI 0.69 to 0.89). Residual analysis of this model indicated no significant deviation from linearity (p = 0.360). The receiver operating characteristic curve demonstrated an area under curve to be 0.88 (0.82 to 0.92), with a positive predictive value of 80.3%. The correlation between CD34+ and XN-HPC showed a strong relationship and good agreement with minimal bias.

Conclusion: The XN-HPC showed good analytical performance. With the increasing requirements for stem cell transplantation, a technically simple and rapid alternative for stem cell enumeration that is sustainable is highly useful.

Keywords: XN-HPC, CD34+, progenitor, leukemia, allogeneic, autologous

Introduction

Hematopoietic stem cell transplantation (HSCT) uses hematopoietic progenitor cells or stem cells (HSC) to replace diseased bone marrow with healthy progenitor cells to repopulate and propagate the bone marrow.[1,2] HSCT is a well-established treatment for high-risk malignant and non-malignant hematologic diseases, immunologic or metabolic disorders, and solid tumours.[3–6] For haematological malignancies, the source of stem cells has shifted from bone marrow collection, which is an invasive procedure, to a much less invasive method in the form of peripheral blood stem cell (PBSC) collection.[7] PBSC transplantation can be summarized into several distinct phases, but the most crucial phase that would allow for successful transplantation is the first phase, which is the mobilization or movement of stem cells from

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the marrow into the peripheral blood induced by timely administration of specific human hematopoietic granulocyte-colony stimulating factors.[8] HSCs used for HSCT in eligible patients can be collected from healthy donors for allogeneic HSCT or, in some instances, the progenitors can be collected from the patients themselves for autologous HSCT. Both procedures require careful consideration by the HSCT transplant team.[7]

The mobilization of stem cells requires close monitoring of stem cell counts that have migrated into the circulation. To ensure the success of the mobilization phase, several authors have recommended that peripheral blood samples be collected daily for CD34+ cell enumeration by flow cytometry analysis until the CD34+ cell count is between 8 and 20 CD34+ cells/μL.[9,10] Donors or patients who do not reach this threshold are considered to have failed PBSC collection and would be counselled for a bone marrow harvest to be performed under general anesthesia. The CD34+ cell dose or the absolute number of CD34+ cells is the best available predictor of graft quality.[11,12]

For a peripheral blood stem cell (PBSC) transplant to be successful, the right amount of HSCs must be infused in order to promote a fast and long-lasting haematologic recovery. The International Society of Hematotherapy and Graft Engineering (ISHAGE) has developed a procedure that relies on flow cytometry to count CD34+ cells for the assessment of circulating HSCs and for determining when to begin apheresis. CD34+ cell count is used as a marker of bone marrow response to stem cell mobilisation accurately that is safe and harmless to the donor.

CD34+ cell count is used to define cell harvest goals and guide growth factor administration and the number of leukapheresis sessions necessary. However, this method is quite costly, as multiple flow cytometry runs must be performed while monitoring the mobilization phase. Our centre uses a threshold of ≥10 cells/μL when deciding whether to proceed with PBSC harvesting for allogeneic and autologous transplantation. Since the first XN model was equipped with the XN-HPC program, many research groups have studied this parameter to ensure its suitability for stem cell enumeration. However, the suggested XN-HPC cutoff values were variable and should be determined for each centre. Sysmex XN analyser uses fluorescence flow cytometry to analyse physiological and chemical properties of cells. It provides the information about cell size, cell structure and cell interior. HPC is counted in the WPC channel of XN analyser. With its unique combination of reagents, WPC channel detects abnormal membrane composition and nuclear content. The lipid membrane composition of immature cells is different from that of mature cells or abnormal blasts. Stem cells' membranes are relatively resistant to permeabilisation by the WPC reagent. As a result, stem cells are medium in size (medium FSC), have a low granularity (low SSC) and relatively low fluorescence intensity (low-medium SFL). In the Sysmex XN analyser, HPCs can be measured in a simple and reliable method within a few minutes without the need for manual gating, pre-treatment or sample washing as compared to immune flow cytometry measurement. However, this requires further validation and comparison with the larger community of XN-HPC users, as this would allow optimization of the timing of apheresis. Therefore, the main aim of this study was to compare HSC enumeration by XN-HPC and CD34+ enumeration by flow cytometry and to determine the correlation and accuracy of its identification.

Materials and Methods

Study Design

This prospective observational study was performed at the National Hematology Referral Centre in Hospital Ampang, Malaysia. Informed consent was obtained before we recruited all healthy Malaysian donors and patients who qualified for autologous hematopoietic stem cell transplantation, regardless of age or gender. Non-Malaysian citizens, and people who were unable or unwilling to give written consent were all excluded from this study.

We recruited patients with Acute Leukaemia, Multiple Myeloma, Hodgkin Lymphoma and non-Hodgkin lymphoma for our allogeneic and autologous stem cell transplantation cases. Peripheral blood stem cells were mobilised following our institution protocol using granulocyte-colony stimulating factor (G-CSF) alone or in combination with chemotherapy. The chemotherapy treatment was followed by administration of G-CSF at a dose of 5 μg/kg/day for patients, and healthy donors were only administered G-CSF at a dose of 10 μg/kg/day. The CD34+ assessment was performed starting from 3 to 4 days after the first administration of G-CSF in healthy donors and after 10–15 days after chemotherapy. Apheresis was started when the quantity of CD34+ cells in PB was at least 20×10^6/L, or in some special circumstances, between 10 and 20×10^6/L, depending on the patient's characteristics.

Peripheral blood for stem cell enumeration was collected preharvest as scheduled by the transplant team and analyzed using the CD34+ cell count and XN-HPC program. This study was reviewed and approved by the Medical Research and Ethics Committee of the Ministry of Health, Malaysia (NMRR-20-2585-57209).

Stem Cell Enumeration by CD34+ Cell Count

Peripheral blood was used for CD34+ HSC enumeration using the BD Stem Cell Enumeration kit on a FACS Calibur flow cytometry analyzer (BD Biosciences, CA, USA) according to the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol.[13] All samples were collected in K2EDTA tubes, stored at room temperature, and processed within 2 hours of collection. The flow cytometry method for stem cell enumeration uses special beads coated with monoclonal antibodies against CD34 and CD45 and a viability dye. Stem cells were identified using low-side scatter (SSC) CD45+ and CD34+ gating strategies. During sample acquisition, direct volumetric control was used to establish the stem cell concentration.

Stem Cell Enumeration by XN-HPC Programme

The same peripheral blood sample was used for HSC enumeration using the XN-HPC program on a Sysmex XN-3000 hematology analyzer (Sysmex Corporation, Kobe, Japan). HSC identification uses a semiconductor laser beam at a wavelength of 633 nm, which is emitted to the blood cells passing through the flow of cells upon acquisition into this channel. This channel, also known as the white blood cell progenitor and pathogenic cells (WPC), is a fluorescence channel that provides information about the cells in the form of forward-scattered and side-scattered light that is presented as a plot that gates for the specific pattern for stem cell identification and enumeration. Forward scattered light hits the cells directly and emits information about the diameter of the cell that translates as the size of the cells, while side-scattered light hits the cell at an angle and emits information about the cytoplasmic properties or complexity, which is usually low for stem cells. Three consecutive runs were performed for each sample to ensure within run precision.

Pre-Study Evaluation of Sysmex XN-HPC Performance

Repeatability of XN-HPC stem cell enumeration was evaluated by measuring five samples comprising three normal subjects and two XN-CHECK control materials, which were tested on the Sysmex XN-HPC ten times consecutively. The average coefficient of variation (CV%) was 19.7% (range 3.7–27.2%). Instrument precision limit was 30%. For stability testing, test samples stored at room temperature and 4 °C were determined by XN-HPC measurements after 0, 2, 4, 6, and 8 hours. Stability test results were assessed using two-way analysis of variance. The samples were stable for at least 8 hours.

Statistical Analysis

Continuous variables are described as means with standard deviations for normally distributed data or medians with a range for skewed data. Categorical variables were described as frequencies and percentages. The Wilcoxon signed-rank test was used to compare the median values of CD34+ and XN-HPC cell counts. The relationship between CD34+ and XN-HPC cell counts was further analyzed using a linear regression model. Passing and Bablok method was used to estimate the slope and intercept with 95% confidence interval (CI). Using Spearman correlation, the correlation coefficient, or r and its 95% confidence interval were determined. To determine the degree of agreement between the two measurements, the Bland–Altman analysis was used. The difference between the two measurements is shown against the average of the two measurements along with the bounds of agreement using the Bland and Altman plots. Ninety-five percent of the mean difference data should fall within the lower and upper limits of the agreement range, which signifies the measurement accuracy. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic accuracy of XN-HPC. The optimal cutoff point was determined using the Youden index. Based on earlier research,[14,15] sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed at the appropriate XN-HPC count that best predicted the CD34+ count at $10.0x10^6$/L and $20.0x10^6$/L.[16]

Results

Study Population

Patients and donors were referred to the Stem Cell Transplant Unit of the Department of Hematology, Hospital Ampang, Malaysia, for allogeneic or autologous peripheral blood stem cell collection and transplantation. A total of 201 mobilised peripheral blood samples were randomly chosen for preharvest processing out of 39 healthy donors and 105 patients who received PBSC transplantation. Thirty were healthy donors and nine were matched unrelated donors. The majority were adults (n = 133) with a few subjects being less than 18 years old (n = 11). The characteristics of the patients and donors and the number of peripheral blood samples in each group are listed in Table 1. The overall median HSC concentrations were not significantly different across subjects. The median CD34+ cell count was 17.33 $x10^6$/L (interquartile range: 0.67–115.67 $x10^6$/L), whereas the median XN-HPC cell count was 13.64 $x10^6$/L (interquartile range: 0.06–152.31 $x10^6$/L).

Correlation and Agreement Studies

The correlation of CD34+ and XN-HPC cell counts in 201 preharvest peripheral blood sample collections is shown in Table 2 and Figure 1. A good agreement was observed between the two methods for all 201 samples. All preharvest samples showed strong correlation (r = 0.77, 95% CI: 0.70–0.82). The regression line had an intercept of 3.45 (2.54 to 4.74) and a slope of 0.78 (0.69 to 0.89). The regression equation was defined as XN-HPC = 3.45 + 0.78 (CD34+) (Figure 1A). The residual analysis of this model indicated no significant deviation from linearity (P = 0.360). The agreement between the two methods was evaluated using Bland–Altman plot (Figure 1B) that showed good agreement with minimal bias between CD34+ and XN-HPC up to a certain range. About 94% of the data points lie within the ±2 SD of the mean of difference. Good agreement and no systematic difference were shown between the CD34+ cell count and XN-HPC at a range of 0 to 15. Thirteen or 6.4% were identified as outliers, and the XN-HPC count differed from CD34+ cell counts by greater than 1 SD above or below the limit of agreement line. For allogeneic preharvest samples, peripheral blood samples from healthy allogeneic donors (N = 42), the correlation strength was moderate (r = 0.69, slope 0.61, 95% CI: 0.40–0.85) (Table 2). A strong correlation was also found in 20 samples collected from multiple myeloma (MM) patients (r = 0.78, slope 1.24, 95% CI: 0.83–1.77), despite a modest underestimation of XN-HPC noted in the Passing–Bablok regression analysis (95% CI of slope: 1.24–0.23) and Bland–Altman plot (95% CI of mean bias: −2.94 to 17.42) (Figure 1).

Receiver Operating Characteristics (ROC) Analysis and Diagnostic Accuracy

Table 3 shows the overall results of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) corresponding to XN-HPC count, which best predicted CD34+ cell count at ≥10×106/L and ≥20×106/L 14,15. The value of area under the curve (AUC) with 95% confidence interval are reported. The overall sensitivity and specificity for XN-HPC count of ≥$10×10^6$/L were 90.7% and 74.2%, respectively, compared to CD34+ cell count of ≥$20×10^6$/L (sensitivity: 89.3%, specificity: 73.5%). Based on the CD34+ cell count by flow cytometry, 93 of the samples were less than $10×10^6$/L. The diagnostic accuracy

Table 1 Characteristics of Patients and Donors, Number of Mobilised Peripheral Blood Samples and Values Distribution of Haemopoietic Stem Cells Using CD34 Positive and XN-HPC Cell Counts

Characteristics	n	Age, Median (Range)	Samples, n	CD34+×10^6/L Median (Range)	XN-HPC×10^6/L Median (Range)	p-value*
All	144	37 (11-71)	201	17.33 (0.67–115.67)	13.64 (0.06–152.31)	0.387
Female	62	40 (26-71)				
Male	82	35 (11-67)				
Allogeneic donors	39	30 (11-67)	42	31.17 (6.33–101.67)	39.07 (5.72–152.31)	< 0.001
Autologous patients	105	40 (12-71)				
Multiple myeloma (MM)	20	57.5 (16-71)	25	25.33 (3.33–115.67)	25.02 (2.51–94.83)	0.083
Lymphoma	79	31 (12-62)	128	12.00 (0.67–97.33)	7.89 (0.06–138.88)	0.004
Leukemia	6	44.5 (22-69)	6	7.17 (1.67–44.67)	14.59 (0.73–87.50)	0.917

Note: *Wilcoxon signed-rank test: Comparison between the median values of HPC and CD34 + counts.

Table 2 Comparison Between XN-HPC and CD34+ Cell Counts

Group	r value[a]	Passing–Bablok Regression*		Bland–Altman Difference Plot (×10⁶/L)	
		Slope (95% CI)	Intercept[b] (95% CI)	Mean Bias** (95% CI)	95% Limits of Agreement (Mean Bias ±1.96 SD)
All	0.766	0.78 (0.69 to 0.89)	3.45 (2.54 to 4.74)	−1.56 (−4.81 to 1.69)	−47.36 to 44.23
Allogeneic donors	0.690	0.61 (0.40 to 0.85)	3.98 (−5.41 to 11.29)	−16.31 (−24.97 to −7.64)	−70.79 to 38.18
Autologous patients					
MM	0.781	1.24 (0.83 to 1.77)	0.23 (−5.35 to 6.50)	7.24 (−2.94 to 17.42)	−41.11 to 55.59
Lymphoma	0.742	0.90 (0.77 to 1.06)	2.83 (2.17 to 4.25)	2.03 (−1.17 to 5.24)	−33.89 to 37.96
Leukemia	0.086	1.12 (−0.23 to 2.58)	2.18 (−24.12 to 16.89)	−11.73 (−50.64 to 27.19)	−84.40 to 60.95

Notes: [a]r value: Spearman correlation. [b]Intercept: 95% Confidence Interval (CI): ×10⁶/L. *Passing–Bablok regression; XN-HPC = Intercept + Slope (CD34+). **Mean bias = XN-HPC - CD34+.

of XN-HPC was assessed using the AUC in the ROC analysis (Figure 2). ROC curves were generated to investigate the ability of preharvest HPC concentrations to predict sufficient CD34+ cell harvest. ROC analysis showed excellent test performance for the preharvest XN-HPC concentration (AUC: 0.86 ≥20/μL CD34+). We also generated ROC curves for preharvest HPC concentrations to predict a sufficient concentration of CD34+ cells for PBSC harvest, which was defined as ≥10×10⁶/L CD34+, as the expected threshold. The test performance was also very good (AUC: 0.88 ranging 0.82–0.92). The Youden index method was used to determine the optimal cutoff point. We identified the cut-off values of the XN-HPC count capable of maximizing its efficiency to be used as a "rule-in" and "rule-out" test for starting apheresis.

Discussion

In the current study, we attempted to establish the correlation between XN-HPC and CD34+ hematopoietic stem cell enumeration after mobilization and whether XN-HPC cut-off values could predict the optimal numbers of CD34+ HSC before PBSC harvest was initiated. We recruited subjects that included both allogeneic donors and candidates for autologous transplantation, as in previous studies.[14,15,17–19] Factors that could influence the yield of stem cells included method of collection, timing of the analysis and storage. To minimize and work around the possible biases, the phlebotomist and operators of both the CD34+ HSC enumeration by flow cytometry and the XN-HPC method were the same person throughout the study. The timing of venipuncture was also standardized in the morning session and samples were analyzed immediately upon collection. Overall, the correlation and ROC analyses showed a strong correlation between the two methods and were comparable to the values previously reported.[17–19] Positive bias was very small at 1.5% and 95% limits of agreement of −47 to

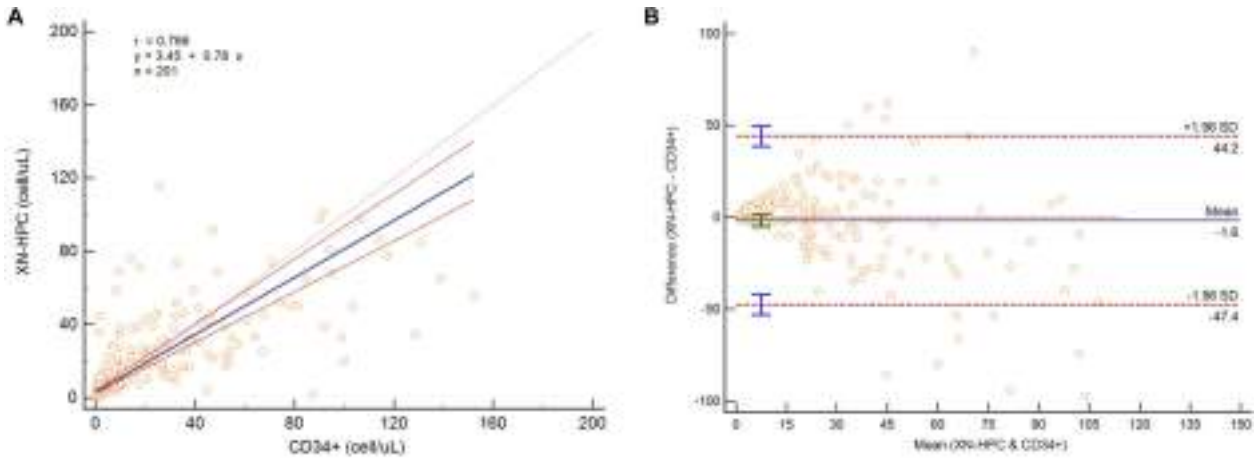

Figure 1 Correlation between XN-HPC and CD34+ cell count analysis using (**A**) Passing and Bablok regression analysis and (**B**) Bland–Altman analysis.

Table 3 Results of Receiver Operating Characteristics Curve Analysis

Total Samples, n	Cut-off CD34+[a]	Number of Positives[b]	AUC (95% CI)	Cut-off XN-HPC	Sensitivity	Specificity	PPV	NPV
All, 201	**≥10.0**	108	0.88 (0.82 to 0.92)	>11.6[c]	90.7	74.2	80.3	87.3
Donor, 42	**≥10.0**	39	0.94 (0.83 to 1.00)	>17.0[c]	82.1	100	100	30.0
MM, 25	**≥10.0**	16	1.00 (NA to NA)	>13.6[c]	100	100	100	100
Lymphoma, 128	**≥10.0**	49	0.85 (0.78 to 0.91)	>11.6[c]	87.8	72.2	66.2	90.5
Leukemia, 6	**≥10.0**	4	0.75 (0.33 to 1.00)	>6.67[c]	75.0	100	100	66.7
All, 201	**≥20.0**	84	0.86 (0.81 to 0.91)	>16.0[d]	89.3	73.5	70.8	90.5
Donor, 42	**≥20.0**	37	0.92 (0.83 to 1.00)	>18.0[d]	81.1	100	100	41.7
MM, 25	**≥20.0**	14	0.97 (0.92 to 1.00)	>13.6[d]	100	81.8	87.5	100
Lymphoma, 128	**≥20.0**	31	0.83 (0.76 to 0.91)	>16.0[d]	90.3	73.2	51.9	95.9
Leukemia, 6	**≥20.0**	2	0.50 (0.00 to 1.00)	>19.3[d]	50.0	100	100	80.0

Notes: [a] Cut-off value of CD34+ cell count (×10^6/L). [b] Number of CD34+ positive samples. [c] Cut-off value of XN-HPC count (×10^6/L) that optimally predict PB CD34+ ≥10.0×10^6/L. [d] Cut-off value of XN-HPC count (×10^6/L) that optimally predict PB CD34+ ≥20.0×10^6/L.

44%. However, the significant difference in HSC enumeration values was notable in the allogeneic donor and lymphoma patient groups (Table 1). For allogeneic donors, Passing–Bablok and Bland–Altman analyses showed that the correlation was good with a negative mean bias of −16.31% indicating a tendency of HSC underestimation using the XN-HPC and 95% limits of agreement of −70.79 to 38.18%. For the lymphoma group, the analyses showed a strong correlation with a positive mean bias of 2.03%, indicating the opposite and 95% limit of agreement of −33.89 to −37.96%.

In the transplant setting, both these potential scenarios would influence the decision on whether to proceed with PBSC harvest or whether further mobilization is required. Therefore, we tested the XN-HPC enumeration values by applying our centre's threshold value of ≥10.0×10^6/L CD34+ HSC, indicating successful mobilization (Table 3). Overall, 53.7% (108 of 201) mobilized peripheral blood samples had a CD34+ cell count ≥10.0×10^6/L by flow cytometry and were identified at a slightly higher XN-HPC cut-off value of 11.6 x10^6/L. ROC curve analysis of XN-HPC count yielded very good accuracy. The sensitivity of this approach was excellent at 90.7%, with high specificity of 74.2%. This indicated that XN-HPC was able to detect and measure CD34+ HSC correctly (Table 3). This was also reflected in the very high positive predictive and negative predictive

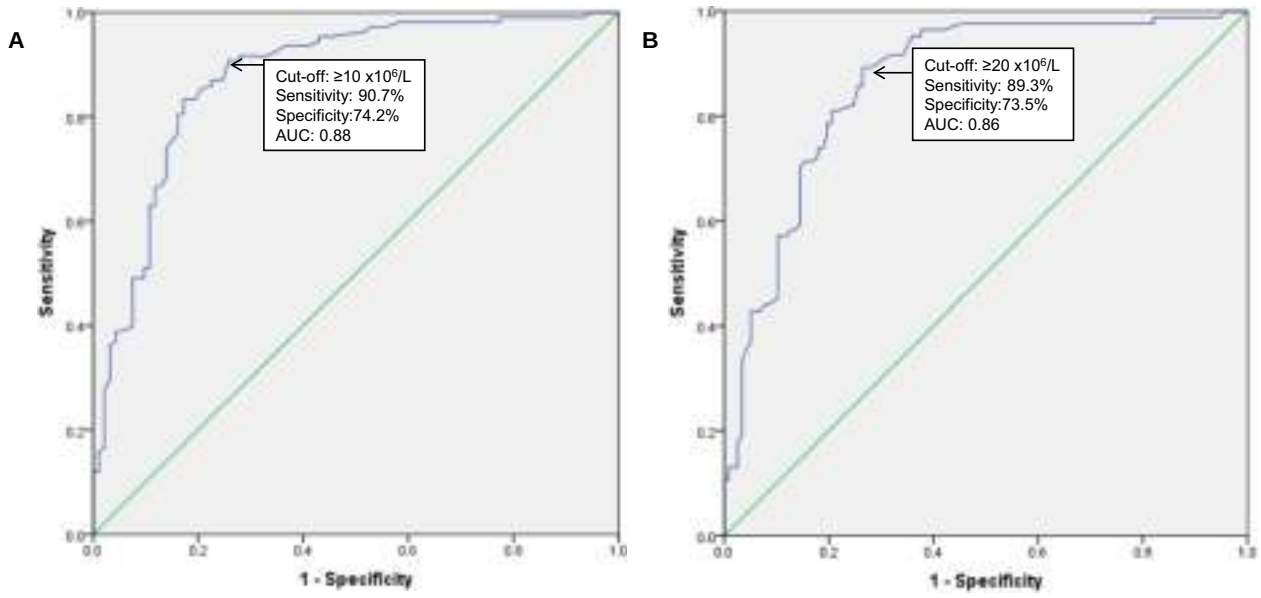

Figure 2 ROC curve for all patients and donors to determine the optimal cut-off for which the HPC concentration can reliably predict CD34+ cell concentration (**A**) ≥10 x10^6/L AUC: 0.88 and (**B**) ≥20 x10^6/L AUC: 0.86.

values of 80.3% and 87.3%, respectively (Figure 2). A few studies have noted significant differences in HSC enumeration in samples from multiple myeloma patients.[15,17] Our findings indicated that although the sample size was small for this group of samples, there was no significant difference between the two methods while the sensitivity and sensitivity were very high. Some authors have indicated caution when using a single cut-off value, as this may cause too many patients to be eligible for harvest before they are adequately mobilized. The laboratory may consider a positive cutoff with high specificity before the initiation of the PBSC harvest. We analysed different XN-HPC cutoff values for each of the different sources of samples from patients who were allogeneic donors or candidates for autologous transplantation. Different cutoff values were derived that maintained high sensitivity and specificity. There was not much difference in the cutoff values, except for the donor category. The highest XN-HPC cut-off was with allogeneic donors at 17.0×10^6/L that could optimally predict CD34+ HSC of $\geq 10.0 \times 10^6$/L but with very good sensitivity and specificity. Autologous samples from leukemia patients showed the lowest cut-off value, but the number of samples for this category was too small.

Overall, our results show a strong correlation with high accuracy. Individual cutoffs should be established for every laboratory that is invested in using the marker for CD34+ enumeration. The use of XN-HPC concentration as a surrogate for CD34+ cell concentration derived from the regression equation may be integrated into the current preharvest workflow with a timely evaluation by CD34+ cell enumeration by flow cytometry. As a surrogate, serial XN-HPC values should be obtained at predetermined time points to increase its specificity. Each transplant centre should determine which patients would be suitable for XN-HPC monitoring and which patients would benefit from CD34+ cell count by flow cytometry. The inherent variability seen across different sample categories, although showing good positive predictive values, may be related to the small number of samples analyzed for each category. The role of previous chemotherapy in autologous samples and its effect on the quality of HSC remain largely unknown. Involvement in the interlaboratory analysis of the same sample is warranted to ensure the robustness of the test platform over time. Most studies agree that the XN-HPC program is fast, simple to use, does not require experienced operators, and is affordable, especially for monitoring purposes.[14,18,19] However, poor agreement between the two methods especially in the clinical decision range and when stem cells were detected at very low concentrations has been reported.[15] It is also crucial that other sources of stem cells can be measured, including marrow, cord blood, and cryopreserved products, especially when matched unrelated donors are not available.

Rapid and durable hematological recovery is dependent on successful peripheral blood stem cell transplantation, which relies on the infusion of an appropriate number of hematopoietic stem cells.[1,20] Therefore, adequately mobilized hematopoietic stem cells in the peripheral blood are crucial before PBSC harvesting is performed. As the main transplant centre in Malaysia, the increasing number of patients brought in for stem cell transplantation to consolidate remission status post-chemotherapy requires a sustainable approach to monitoring stem cell concentration before PBSC harvest. Therefore, incorporating this method for CD34+ monitoring would improve the efficiency of transplant services in general and easing financial constraints while maintaining the availability of the service. Moving forward, a testing algorithm that incorporates XN-HPC in the monitoring of HSC mobilized as well as establishing triggers for CD34+ cell enumeration by flow cytometry will be proposed to evaluate the effectiveness of certain cutoff values for XN-HPC for allogeneic and autologous transplantation.

Conclusion

Our study confirms a strong correlation between XN-HPC count and $CD34^+$ cell count and could be a useful surrogate test to assess optimal timing for PBSC collection. It can be used as an alternative method for CD34+ cell count. However, a workflow that would incorporate the XN-HPC count in daily peripheral blood cell count monitoring during mobilization that is used for screening and confirmation with CD34+ cell count at least one day prior to or on the day of the intended peripheral blood stem cell harvest would greatly increase the sustainability of bone marrow transplant unit services. Further prospective studies are recommended to evaluate the effectiveness of a working algorithm that incorporates XN-HPC cell counts in both healthy donors and autologous patients.

Ethics Approval and Consent to Participate

This study was approved by the Medical Research and Ethics Committee of the Ministry of Health Malaysia (NMRR-20-2585-57209). Study participants provided informed consent to participate. Research was conducted in line with the Belmont Report and the Declaration of Helsinki.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study. None of the authors were reimbursed for conducting this study.

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Management of Congenital Methemoglobinemia in the Perioperative Setting: A Case Report and Review of Current Literature

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CASE REPORT

Management of Congenital Methemoglobinemia in the Perioperative Setting: A Case Report and Review of Current Literature

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Background: Methemoglobin is an altered state of hemoglobin where iron in hemoglobin is oxidized and incapable of binding oxygen; leading to complications such as cyanosis, dyspnea, headache, and heart failure. Methemoglobinemia can be congenital or acquired. Congenital methemoglobinemia is a rare disease and its worldwide incidence is unclear. We recently encountered the first documented case of congenital methemoglobinemia at our institution, necessitating perioperative care.

Case Presentation: In the present case, a 22-year-old man with congenital methemoglobinemia underwent general anesthesia for dental extraction. The surgeon was informed to avoid local anesthetics and oxygenation was performed with FiO_2 of 1.0. Arterial blood gas analysis showed a PH of 7.337, PaO_2 of 302 mm Hg, $PaCO_2$ of 44 mm Hg, oxyhemoglobin level of 63.4%, and methemoglobin level of 37.8%. The patient had a stable course. No methylene blue therapy was required, although cyanosis was observed during surgery.

Conclusion: In summary, though rare, congenital methemoglobinemia poses fatal risks during surgery. Its management involves preoperative recognition and optimization, oxygenation status, multidisciplinary care, avoiding precipitating or oxidizing agents, discussing treatment options, maintaining cardiopulmonary stability, and ensuring perioperative safety measures with the medical team.

Keywords: methemoglobinemia, general anesthesia, perioperative management, cyanosis, methylene blue

Introduction

Methemoglobin (MetHb) is an altered state of hemoglobin (Hb) containing iron in the Ferric (Fe^{3+}) state rather than Ferrous (Fe^{2+}). Ferric iron cannot bind and transport oxygen. Thus these patients can develop functional anemia and tissue hypoxia. MetHb is normally present in less than 1% of the blood concentration.[1] With higher concentrations, symptoms develop.

Methemoglobinemia can be congenital and is usually rare. It can be attributed to either a cytochrome b5 reductase deficiency or the presence of hemoglobin M disease.[2]

Acquired methemoglobinemia is however more common and unfortunately more severe. This condition can arise from using different pharmacological agents including nitrates, and local anesthetics such as prilocaine, dapsone, and nitroglycerine (Box 1).[3]

We describe the perioperative management of a patient, diagnosed with congenital methemoglobinemia, who required general anesthesia for dental extraction.

Case Report

Background

We report a 22-year-old (98 kg bodyweight, 166 cm tall, BMI 35.6) Middle Eastern male, posted for elective extraction of carious wisdom teeth under general anesthesia. He was known to have congenital methemoglobinemia as well as some

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Box 1 Common Agents Causing MetHb

Chemicals
Aniline dyes
Fava Beans
Fumes (Wood, Plastic, Automobile exhaust)
Ginkgo biloba
Herbicides
Mothballs
Nitrates[a]
Octane Boosters
Petrol
Well water
Medications
Acetaminophen[a]
Acetanilide
Benzocaine[a]
Bismuth subnitrate
Chloramine
Chloroquine
Copper sulfate
Dapsone
Flutamide
Lidocaine[a]
Metoclopramide[a]
Nitric Oxide[a]
Nitromethane
Nitrofurans

(*Continued*)

Box 1 (Continued).

Nitroglycerin[a]
Nitroprusside[a]
Paraquat
Phenacetin
Phenazopyridine
Phenytoin[a]
Prilocaine[a]
Primaquine
Rasburicase
Silver nitrate
Sodium valproate[a]
Sulfasalazine
Sulfonamides[a]
Zopiclone

Notes: [a]Common agent used in the operating room. Reprinted from Barash M, Reich KA, Rademaker D. Lidocaine-induced methemoglobinemia: A clinical reminder. *J Am Osteopath Assoc*. 2015;115(2):94–98. Creative Commons.[3]

learning disability. Of note, two years prior, he underwent dental extraction in a dental clinic under local anesthesia, following which, he developed severe cyanosis later in his home, requiring hospital admission and intensive care unit management.

The patient also has had multiple previous visits to our hospital emergency department mainly with cyanosis and associated symptoms. Most of the ED visits had culminated in him receiving methylene blue and hydroxycobalamin before being discharged. A hematologist and cardiologist's opinion had been documented in his health records. His co-existing learning disability suggested a diagnosis of Autosomal recessive congenital methemoglobinemia. Echocardiography had ruled out congenital cyanotic heart disease.

Preoperative Assessment and Optimization

Armed with the available history, our focus was on the patient's cardio-respiratory fitness. In the pre-anesthesia visit for his current surgery, our patient reassured us that he could comfortably engage in routine physical activities, including walking and climbing stairs, without experiencing headaches, palpitations, or dyspnea.

On examination, he had visible bluish discoloration of his fingers and lips. The airway assessment revealed Mallampati grade II, adequate mouth opening, and normal neck movement. Oxygen saturation (SPO2) at the time was measured only 87% on room air. Blood investigations showed secondary polycythemia (Figure 1), Glucose-6-Phosphate Dehydrogenase (G6PD) within normal levels, and methemoglobin at around 3.9–7.1% (Figure 2).

A Transthoracic Echocardiography reported mild aortic regurgitation and trivial tricuspid regurgitation.

He was referred to a hematology and genetic clinic for whole exome sequencing.

The hematologist opined that the patient is fit for surgery and to avoid venesection for polycythemia as it will worsen his condition.

Figure 1 Patient had secondary polycythemia with Hb values ranging from 17.5 to 18.5 gm/dl.

Intraoperative Management

On arrival in the operating room, baseline SPO2 was 84%. Following five minutes of preoxygenation with 100% oxygen, induction of general anesthesia was performed with calculated doses of fentanyl (2 mcg/kg), propofol (2mg/kg), and rocuronium (1mg/kg).

A 6.5 mm ID nasal endotracheal tube was inserted uneventfully and anesthesia was maintained with 100% oxygen, sevoflurane, and controlled ventilation (volume controlled, Tidal volume 450 mL, Respiratory rate 12/min, Peak airway pressure 23 mmHg, Positive end-expiratory pressure 5 mm Hg). SPO2 marginally improved to 87% on controlled ventilation and oxygenation.

An arterial line was inserted post-induction for frequent blood sampling for partial pressure of oxygen (PO2), patient acid-base balance, and methemoglobin level measurement. Invasive blood pressure monitoring was used to rapidly determine significant hemodynamic changes. The surgical team was requested to avoid lidocaine. Methylene blue was kept ready in case.

Methemoglobin level post induction was 37.8% and SPO2 was at 87% and considered inaccurate. The procedure was uneventful with no desaturation from baseline or acidosis in blood gases.

Postoperative Management

Emergence from general anesthesia and extubation was smooth. The patient was moved to the Post Anesthesia Care Unit (PACU) and admitted inpatient for continued observation.

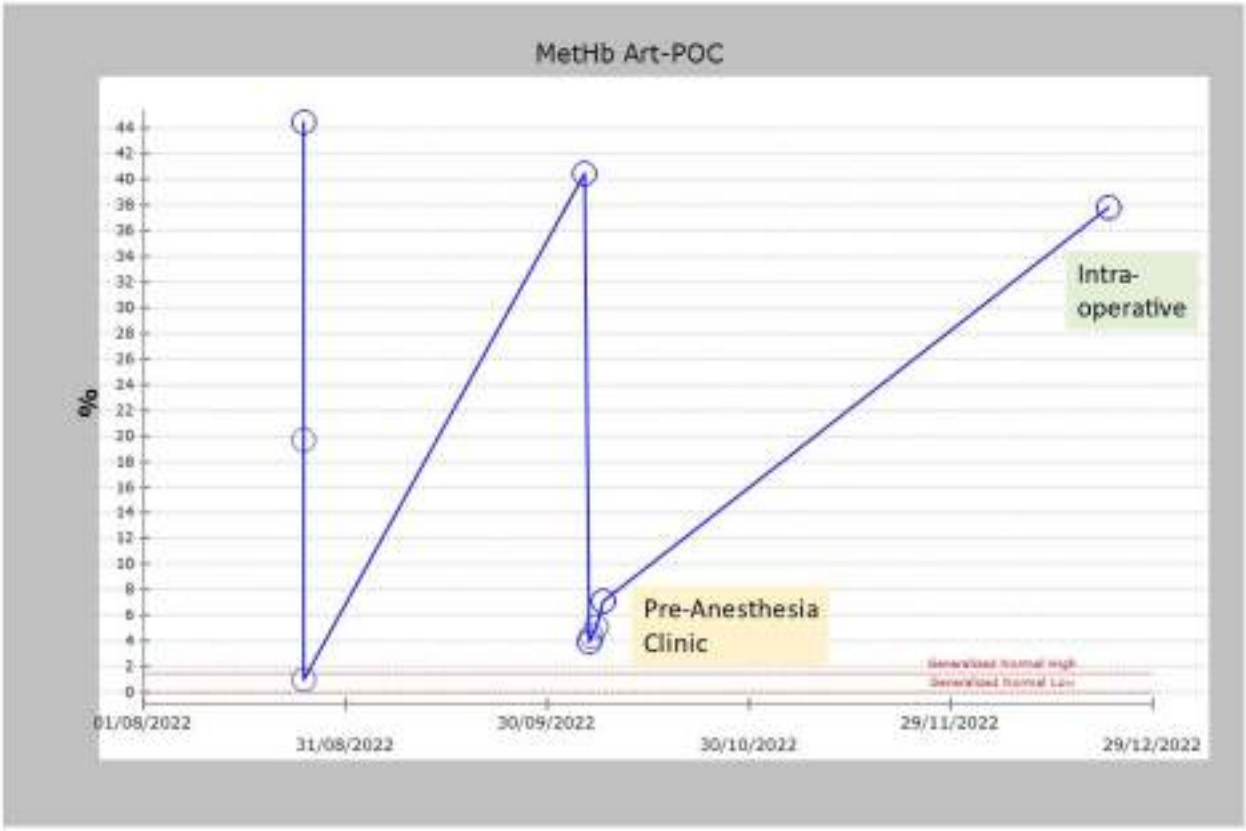

Figure 2 Patient had varying concentrations of MetHb with values from 1 to 44.5%.

Discussion

Methemoglobin refers to a variant of hemoglobin that undergoes oxidation, leading to a transition in its heme iron configuration from the ferrous fe2+ to the ferric fe3+ state. This state of hemoglobin lacks oxygen-carrying capacity and in good health is normally present in less than 1% of the blood concentration (the normal range has been described as 1–3% in some sources).[1]

During red blood cell metabolism, methemoglobin is formed and converted back to its normal ferrous state at low levels. The process of methemoglobin production and reduction is typically balanced to uphold a steady-state level, which is around 1% of the total hemoglobin content (Figure 3).

Methemoglobinemia describes an imbalance in this equilibrium leading to Methemoglobin levels above the normal 1–3%. Hemoglobin deoxygenation, reactions with endogenous free radicals and exogenous chemicals can increase methemoglobin levels. Causes can be congenital or acquired (Table 1 and Box 1).[4]

The failure of methemoglobin to effectively bind oxygen results in the inability to deliver oxygen to tissues. This condition presents a spectrum of symptoms and signs, ranging from simple cyanosis and the distinctive chocolate brown blood (10 to 20% MetHb concentration) to tachypnea, confusion, and syncope (MetHb concentration>30%).[4] Central nervous system hypoxia with seizure and or coma, metabolic acidosis with dysrhythmia occurs for MetHb concentration more than 50% (Table 2).

Under normal physiological conditions, red blood cell enzyme cytochrome b5 reductase maintains low levels of methemoglobin. Hence, the primary cause of inherited methemoglobinemia is a congenital deficiency in cytochrome b5 reductase and is inherited in an autosomal recessive pattern.[2] Hemoglobin M disease is the other form of congenital methemoglobin and is inherited as an autosomal dominant defect.[5,6] In Hemoglobin M disease, a mutation in the gene coding for one of the globin chains results in a substitution of a tyrosine amino acid for either the proximal (F_8) or the distal (E_7) histidine amino acid in the α, β, or γ chains. This mutation stabilizes the iron in Fe^{3+} form. Most individuals with congenital methemoglobinemia show no symptoms apart from cyanosis.

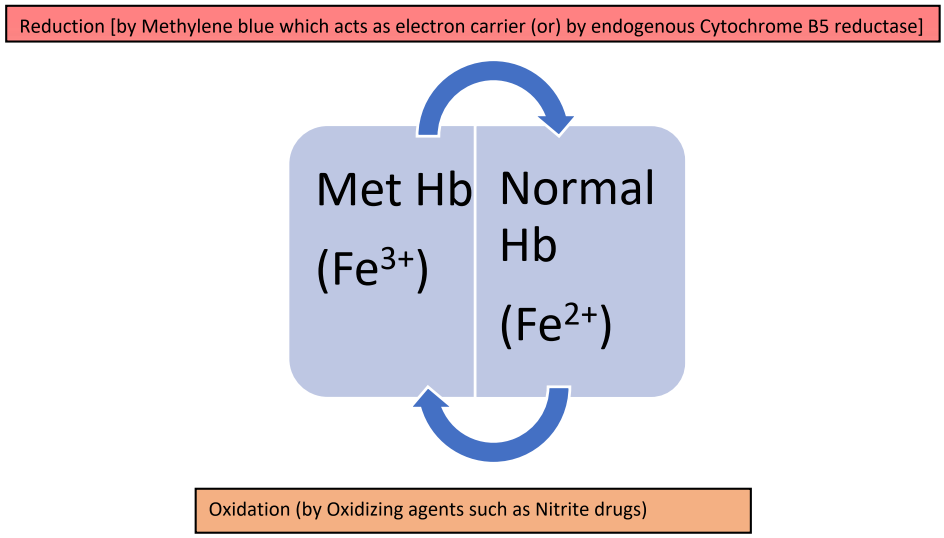

Figure 3 Equilibrium of Met Hb and Normal Hb in the body.

An alternative pathway for methemoglobin reduction, which is not physiologically active, uses nicotinamide adenine dinucleotide phosphate NADPH methemoglobin reductase. NADPH is generated by glucose 6 phosphate dehydrogenase G6PD and this pathway is only activated by extrinsic acceptors such as methylene blue. This requirement of G6PD explains why Methylene Blue therapy is ineffective in individuals with G6PD deficiency.[7]

Congenital methemoglobinemia has three primary genetic causes. The most frequent cause is a deficiency in the CYB5R3 enzyme (autosomal recessive disorder). Following that, two other causes are hemoglobin M disease (autosomal dominant disorder) and cytochrome B5 deficiency. Although congenital methemoglobinemia due to cytochrome b5 reductase deficiency is exceptionally rare, but the actual incidence is unknown. Interestingly, it appears to occur more frequently among Siberian Yakuts,

Table 1 Causes of Congenital Methemoglobinemia

Autosomal Recessive	**Autosomal Dominant**
Cytochrome b5 reductase (CYB5R)deficiency (Congenital Methemoglobinemia)	Hemoglobin M disease
Subtypes: 1. Congenital Methemoglobinemia Type I (enzyme defect in erythrocytes) 2. Congenital Methemoglobinemia Type II (enzyme defect in all cells)	Subtypes: 1. Boston 2. Fort Ripley 3. Hyde Park 4. Iwate 5. Kankakee 6. Osaka 7. Saskatoon

Table 2 Symptoms and Signs in a Patient with Methemoglobinemia (Adopted from Ludlow et al).[2]

% MetHb	**Symptoms**
<15	Generally Asymptomatic
15–30	Cyanosis, anxiety, light-headedness, fatigue, headache
30–50	Tachypnea, confusion, syncope
50–70	Seizures, arrhythmias, metabolic acidosis, coma
>70	Death

Athabaskans, Eskimos, and Navajo populations.[8,9] These CYB5R3 deficiencies come in two types: type I, which affects only red blood cells, and type II, which occurs in all tissues. Patients with type I disease typically experience mild symptoms and have a normal lifespan. In contrast, those with type II disease exhibit cyanosis (bluish skin due to lack of oxygen), along with developmental delay, intellectual disability and other neurological manifestations, and have a significantly shorter lifespan.[1,10]

Acquired methemoglobinemia can range from severe to even potentially fatal depending on the plasma level. It can be a medical emergency and the diagnostic clues include cyanosis; respiratory or neurologic symptoms out of proportion to pulse oximetry; dark red brownish to blue blood that does not turn red with oxygenation; and low pulse oximetry that does not improve with oxygen. These symptoms generally occur with a methemoglobin level of 10% and a level of more than 30% can be life-threatening. Acquired methemoglobinemia may be triggered by various oxidizing agents such as dapsone, chloroquine, metoclopramide, benzocaine, lidocaine, prilocaine, nitric oxide, nitroglycerin, and others (Box 1).[3,11–13]

Methylene blue is often administered (1–2mg/kg IV) to treat patients with methemoglobinemia however it should be avoided in patients with G6PD deficiency due to the risk of hemolysis. A previous study found that methylene blue did not improve methemoglobinemia in patients with Hb M.[14] Therapy with vitamin C can be considered when methylene blue is not indicated. Prophylactic preoperative methylene blue administration in a patient with congenital MetHb lowered the methemoglobin level significantly. This led to a notable rise in oxygen saturation, providing a greater safety margin against hypoxemia during the perioperative period. Alternatively, Hyperbaric oxygen (HBO) therapy was found an effective treatment in MetHb. HBO therapy inhibits the oxidation of hemoglobin by nitrite and reduces MetHb levels by approximately 8% per hour.[1,15]

Inhalation of high concentrations of oxygen can be also used to treat methemoglobinemia and high arterial oxygen pressure should be maintained to minimize tissue hypoxia during induction of general anesthesia. Additionally, blood transfusion or exchange transfusion should be considered in patients with severe MetHb exceeding 70%.[6,16,17] A Summary of recommendations for perioperative management of congenital methemoglobinemia is given in Figure 4.[18]

There have been few reported cases of perioperative management of congenital methemoglobinemia in the literature. In this report, we have also summarized all the reported cases of congenital methemoglobinemia and anesthesia management (Table 3).

In our patient MetHb concentration was 37.8% and pH 7.337, PaO2 302 mm Hg, PaCO2 44 mm Hg, and oxyhemoglobin level of 63.4%. Oxygenation and ventilation with FiO2 at 100% were maintained and the intraoperative vital signs such as blood pressure and heart rate remained stable. Repeated blood gases were taken during the procedure to follow MetHb level and acid-base balance to decide methylene blue utilization (first line).

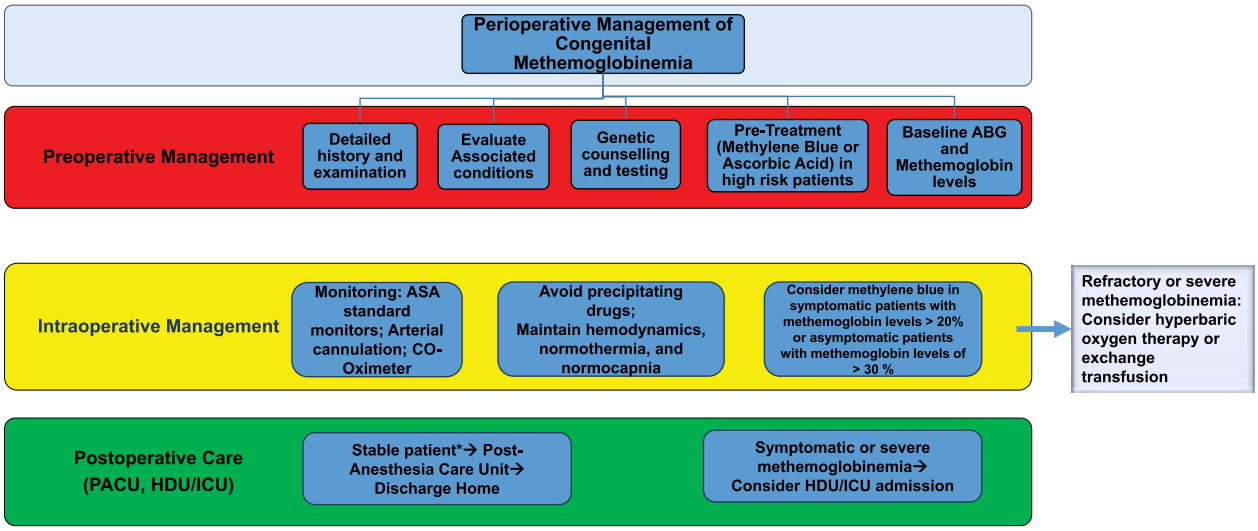

Figure 4 Recommendations for Perioperative Management of Congenital Methemoglobinemia.
Notes: *Stable patient refers to an asymptomatic, hemodynamically stable patient. Such patients can be monitored in the PACU with frequent measurement of methemoglobin levels and standard care.

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Table 3 Summary of Reported Cases of Perioperative Management of Congenital Methemoglobinemia

Author	Age/Sex	Associated Conditions	Surgery	Anesthetic Management	Postoperative course	Outcome
Chisholm et al[19]	24 years old/ Female	Nil	Evacuation of retained product of conception	Anesthesia was induced with intravenous propofol and fentanyl and maintained with 50% nitrous oxide in oxygen with incremental propofol boluses.	Uneventful	Discharged home the next day
Baraka et al[20]	22 years old/ Male		Turbinectomy	Arterial cannula inserted, then lidocaine (1mg/kg) was administered intravenously. The patient developed sudden unconsciousness, and apnea, with severe cyanosis and desaturation (79%). 100% oxygen and methylene blue (1mg/kg) were administered. The patient regained consciousness but the surgery was cancelled.	Stable course	Not reported
Maurtua et al[21]	33 years old/ Female	Nil	Laparoscopic excision of a right rudimentary fallopian tube and hysteroscopy	Arterial line was inserted, followed by intravenous induction with fentanyl, lidocaine, propofol and atracurium	Stable perioperative course, Arterial blood gas (ABG) taken in the recovery room showed no change in MetHb in levels from the baseline.	Not reported
Baraka et al[22]	26 years old/ Male	Nil	Turbinectomy	Arterial line with baseline blood gas sampling, prophylactic methylene blue 1% administered at a dose of 1mg/kg intravenously, followed by IV induction with lidocaine (1mg/kg), propofol (2mg/kg) and rocuronium (0.6mg/kg). Anesthesia was maintained with isoflurane 1 to 2% in 50% of oxygen.	Uneventful course. MetHb fraction was 0.01 postoperatively and increased to 0.026 on the second day to reach 0.094 on the fifth day.	Not reported
Sharma et al[23]	9 years old/ Male	Osler-Weber-Rendu syndrome and bilateral pulmonary arteriovenous malformation	Cerebral angiography	Radial artery cannulated, followed by intravenous (IV) induction with thiopentone sodium (150 mg), and rocuronium (25 mg). Inhalational anesthesia was maintained with 0.8–1% isoflurane. Reversed with IV neostigmine 1.5 mg and atropine 0.6 mg before extubation.	Stable postoperative period.	Not reported
Melarkode et al[14]	60 years old/ Female	Nil	Mastectomy	Arterial line was inserted before anesthesia, induction with IV fentanyl and propofol. Inhalational anesthesia was maintained with Isoflurane.	Uneventful perioperative course	Discharged home two days after the surgery.
Lin et al[6]	35 years old/ Female	Nil	Uterine myomectomy	Following preoxygenation with 100% oxygen, anesthesia was induced with IV midazolam (2.5 mg), fentanyl (75 mcg), lidocaine (50 mg), propofol (2mg/kg) and rocuronium (50 mg). Extubated at the end of the surgery	Smooth recovery.	Discharged home two days later.

Champigneulle[24]	78 years old/ Male	Nil	Aortic valve replacement	General anesthesia was induced with the target-controlled infusion of propofol and remifentanil and a bolus of atracurium.	Extubated a few hours after the surgery.	Discharged from the ICU on day 2 and from the hospital on day 13 without complications.
Gupta et al[25]	26 years old/ Female	Nil	Emergency cesarean section	Spinal anesthesia with 0.5% bupivacaine (2mL).	Stable postoperative course.	Not reported
Yin et al[26]	22 years old/ Female	Nil	Elective induction of Labor	Remifentanil as intravenous labor analgesia, at an infusion of 0.025 mcg/kg/min with a 25 mcg bolus and continuous methemoglobin saturation (CO-Oximeter) monitoring.	Smooth recovery	Discharged home on postpartum day 2.
Ri et al[4]	32 years old/ Male	Hb M disease	Thyroidectomy	Arterial line was inserted followed by IV induction with propofol (200 mg) and rocuronium (90 mg). Anesthesia was maintained with 1.5–2% sevoflurane with FiO2 at 0.6. Target remifentanil set at 2–3 ng/mL IV. Frequent ABG samples taken intraoperatively. Twenty minutes post-induction, SpO2 was 65–75% and cyanosis was observed. FiO2 increased to 1.0. Extubated after two hours of surgery.	Received oxygen at 6 L/min via face mask in the recovery and weaned to room air later.	Uneventful postoperative course and discharged home on day 5 after surgery.
Choi et al[27]	15 years old/ female	Nil	Dental extraction	Remifentanil and propofol infusion started at 0.5 mcg/kg/hr and 100 mcg/kg/min respectively to facilitate arterial cannulation, followed by an induction dose of propofol (150 mg) without neuromuscular blockade. Anesthesia was maintained with isoflurane inhalation and IV remifentanil. CO-Oximeter used intraoperatively for continuous measurement of MethHgb along with cerebral oximetry. Received 4 mg each of dexamethasone and ondansetron as well as 50 mcg of fentanyl intraoperatively. Extubated uneventfully at the end of the surgery	Placed on 6 L/min of oxygen by the face mask and monitored for four hours postoperatively.	Discharged home on the same day of surgery
Karimbanakkal et al[28]	Middle-aged, Male	Diabetes, hypertension, coronary artery disease, post-renal transplant	Parathyroidectomy	After preoxygenation with 100% oxygen, induced with IV etomidate (16 mg), fentanyl (100 mcg), and atracurium (40 mg). Anesthesia was maintained with sevoflurane and IV fentanyl 50 mcg/hr. Arterial line inserted post-induction. Extubated after given reversal. Maintained stable hemodynamic throughout the surgery	Shifted to intensive care unit post-operatively and placed on 4–6 L/min oxygen via Hudson mask.	Not reported

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The routine use of local anesthetic administration was prohibited and no Methylene Blue therapy was needed. Postoperatively, the MetHb level was still 37.8%. We admitted the patient for 24 hours for post-operative observation. He remained stable and was discharged home a day after the procedure. Genetic testing later revealed homozygosity for the cytochrome b5 reductase-3 enzyme leading to cytochrome b5 reductase deficiency.

Conclusion

In conclusion, although congenital methemoglobinemia is rare, it is still life-threatening and therefore warrants strong perioperative consideration. Patients with congenital or inherited methemoglobinemia pose a challenge with respect to perioperative management of oxygenation and global tissue perfusion.

The avoidance of potential oxidizing agents as well as the availability of emergency treatment modalities such as methylene blue or exchange transfusion and hyperbaric oxygen therapy should be discussed. A card with a list of contraindicated medications could be given to the patient to prevent the patient's future exposure and disease exacerbation.

Intraoperatively the anesthesiologist should discuss the safety precautions with the nursing and surgical team. The use of oxidizing agents must be avoided and oxygen carrying capacity must be maintained by ensuring high oxygen concentration in the inhaled gases. Methylene blue can be given prophylactically or as therapy, if the patient has a normal G6PD level. The decision to treat or not is ideally guided by clinical judgment and serial blood gases to check MetHb level, oxyhemoglobin, and acid-base balance can be used to support clinical findings. Blood transfusion or exchange transfusion can also be considered. A hyperbaric oxygen therapy chamber within the hospital facility might also be a great advantage.

Patient Consent

The patient provided written consent for the publication.

Ethics Approval

This case report was reviewed and approved by the Medical Research Centre of Hamad Medical Corporation with the ID: MRC-04-23-423.

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Disclosure

The authors report no conflicts of interest.

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ORIGINAL RESEARCH

Specific Mutation Predict Relapse/Refractory Diffuse Large B-Cell Lymphoma

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Background: The application of rituximab has significantly enhanced the overall survival rates in patients with diffuse large B-cell lymphoma (DLBCL). Regrettably, a significant number of patients still progress to relapse/refractory DLBCL (rrDLBCL).

Methods: Herein, we employed targeted sequencing of 55 genes to investigate if gene mutations could predict the progression to rrDLBCL. Additionally, we compared the mutation profiles at the time of DLBCL diagnosis with those found in rrDLBCL cases.

Results: Our findings highlighted significantly elevated mutation frequencies of *TP53, MEF2B* and *CD58* in diagnostic biopsies from patients who progressed to relapse or refractory disease, with CD58 mutations exclusively observed in the rrDLBCL group. In assessing the predictive power of mutation profiles for treatment responses in primary DLBCL patients, we found that the frequency of *CARD11* mutations was substantially higher in non-response group as compared with those who responded to immunochemotherapy. In addition, we revealed mutations in *HIST2H2AB, BCL2, NRXN3, FOXO1, HIST1H1C, LYN* and *TBL1XR1* genes were only detected in initial diagnostic biopsies, mutations in the EBF1 gene were solely detected in the rrDLBCL patients.

Conclusion: Collectively, this study elucidates some of the genetic mechanisms contributing to the progression of rrDLBCL and suggests that the presence of *CD58* mutations might serve as a powerful predictive marker for relapse/refractory outcomes in primary DLBCL patients.

Keywords: targeted sequencing, mutation profile, relapse/refractory disease, diffuse large B-cell lymphoma

Introduction

Diffuse large B cell lymphoma (DLBCL) represents the most prevalent form of non-Hodgkin lymphoma (NHL), which accounts for 25–30% of all NHL cases with an annual incidence rate of 5.6 per 100,000 individuals.[1,2] According to the cell of origin (COO) classification, DLBCL is divided into germinal center B-cell (GCB) and activated B-cell (ABC) subtypes. These subtypes exhibit distinct clinical outcomes, with the ABC subtype associated with an inferior prognosis.[3] The standard first-line treatment for DLBCL comprises a combination of cyclophosphamide, doxorubicin, vincristine and prednisolone, augmented by rituximab (R-CHOP) immunochemotherapy. Although the incorporation of rituximab has significantly improved the prognosis of DLBCL patients, it has also led to the emergence of specific resistance mechanisms.[4] About 40% of patients with lymphoma eventually develop a relapse or refractory status.[5] The prognosis for patients with relapse or refractory DLBCL (rrDLBCL) remains inferior.[6] Approximately 50% of patients with rrDLBCL achieve a response to second-line chemotherapy, and about half may undergo autologous hematopoietic stem-cell transplantation in certain clinical settings. Despite these interventions, 60% to 70% of these patients experience disease progression within 3 years following transplantation.[6–8] Moreover, the median survival time for individuals with primary and refractory DLBCL is brief, ranging from only 5–7 months.[6] Thus, elucidating the mechanisms driving the relapse or refractory progression of DLBCL is of critical importance.

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Up to now, several studies have investigated the mutation profiles of primary central nervous system lymphoma (PCNSL) and rrDLBCL. For instance, Morin et al[9] explored the genetic landscapes of rrDLBCL and identified *TP53, FOXO1, MLL3, CCND3, NFKBIZ* and *STAT6* as key candidate genes associated with therapeutic resistance. Similarly, Greenawalt et al[10] demonstrated that mutations in *CREBBP* and *BCL2* genes were more prevalent in rrDLBCL compared to the diagnostic biopsies. Rushton et al[11] found 6 genes, namely *KMT2D, TP53, CREBBP, FOXO1, NFKBIE* and *MS4A1* (CD20), showed significant mutation enrichment at relapse in the circulating tumor DNA (ctDNA) samples of rrDLBCL compared with an independent primary cohort. However, there is a scarcity of research focusing on the mutation characteristics in the diagnostic biopsies that can predict the relapse/refractory outcomes of DLBCL.

In this study, we aimed to determine whether the mutation profiles can serve as predictive markers for relapse/refractory outcomes of DLBCL. Additionally, we compared the mutation profiles observed at the time of DLBCL diagnosis with those found in rrDLBCL patients.

Materials and Methods

Patient Information

This study included 96 lymph node samples from 64 primary DLBCL (before treatment) and 32 recurrence DLBCL patients (after treatment). Among these, 2 patients had both primary and relapse samples. The samples were collected from November 2011 to January 2021 at the Peking University Third Hospital (Beijing, China). Primary refractory DLBCL was defined as disease progression during initial R-CHOP regimen without achieving a complete remission (CR) or relapse within 6 months after a transient CR post-initial therapy. Relapsed DLBCL was defined as DLBCL that reappeared after a CR lasting more than 6 months. For comparison purposes, the 64 primary DLBCL patients were divided into DLBCL group (n=46) and rrDLBCL group (n=18) according to the late progression. Additionally, they were categorized into the response group (n=14) and non-response group (n=50). All patients received initial immunochemotherapy with or without radiotherapy or stem cell transplantation. This study got the approval of the Institutional Review Board of the Peking University Third Hospital Medical Science Research Ethics Committee (no. LM2020220) and was carried out in accordance with the Declaration of Helsinki.

Targeted Panel Sequencing

Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) or fresh tissues using the Maxwell® RSC DNA FFPE kit (Promega, Madison, Wisconsin, USA), referring to the manufacturer's descriptions. Targeted panel sequencing, encompassing 55 genes related to hematological malignancies (Supplementary Table 1), was performed on a NovaSeq platform (Illumina, San Diego, CA, USA). DNA quantity was determined using a Nanodrop 8000 UV–Vis spectrometer (NanoDrop Technologies, Wilmington, DE, USA), Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA), and 2200 TapeStation Instrument (Agilent Technologies, Santa Clara, CA, USA). Library was constructed by lymphoma-associated gene mutation detection kit (Shanghai Rightongene Biotechnology Co., Ltd, Shanghai, China). The paired-end reads were aligned to the Human Genome Reference Consortium build 37 (GRCh37) using BWA (version 0.5.9-tpx). Samtools (v0.1.18), picard (v1.93) and GATK (v4.1.4.0) were used for BAM file handling, local realignment, base recalibration and calling variants, respectively. Mutations in the coding region were annotated using the Annovar software (version 2017–07-17).

Mutation Analysis

Variants, including single nucleotide variations (SNVs) and insertions/deletions (Indels), were screened by Shanghai Rightongene Biotechnology Co., Ltd. (Shanghai, China) based on the following filtering conditions: (1) SNVs or Indels with a mutation allele frequency (MAF) ≥ 0.001 in databases of 1000 genomes project,[12] 1000 genome East Asian, ExAC all or ExAC East Asian and genomAD[13] were removed; (2) SNVs or Indels with a variant allele frequency (VAF) $\geq 5\%$ were retained; (3) variants listed in dbSNP (v147) and existing in the COSMIC database were retained; (4) SNPs or Indels including stopgain, stoploss, frameshift, nonframeshift and splicing site alterations were retained; (5) missense mutations meeting the following criteria were retained: SIFT score ≤ 0.05, Polyphen2_HVAR_pred score ≥ 0.447 and

CADD score > 4. "Maftools" package (version 2.2.10) of the R software[14] was used to generate the horizontal histogram illustrating the mutated genes.

Statistical Analysis

The differences in age, sex, COO classification, clinical stage, IPI (international prognostic index), LDH (lactate dehydrogenase) level, ALB (albumin) level, β-macroglobulin, HGB (hemoglobin) level, ESR (erythrocyte sedimentation rate) level, Ca+ level and DPL (double-protein-expression lymphomas) between the two groups were analyzed using Fisher's exact tests. Kaplan-Meier (K-M) curves with Log rank tests were used to analyze the relationship between the mutations and the overall survival of DLBCL patients. Multivariate logistic regression analysis was conducted to assess the factors influencing the response to immunochemotherapy, with a p-value < 0.05 in univariate analysis serving as the threshold for inclusion. p-value < 0.05 was thought as significant difference.

Results

Mutation Characteristics of the Diagnostic Biopsies from Patients Who Developed to rrDLBCL

First, we described the mutation profiles of primary DLBCL patients, who were further divided into rrDLBCL and DLBCL groups, to explore whether certain mutation characteristics could predict relapse or refractory status of DLBCL. As shown in Table 1, the Ann Arbor stage and IPI in the rrDLBCL group were significantly elevated compared with the DLBCL group. Also, we compared the mutation profiles between these groups (Figure 1). The results showed significant differences in the mutation frequencies of *TP53, MEF2B* and *CD58*, especially noting that *CD58* mutations were exclusively detected in the rrDLBCL group (Figure 2A). However, no significant relationship was found between the mutations in *TP53, MEF2B, CD58* and the overall survival of DLBCL patients

Table 1 Clinicopathologic Features of Primary DLBCL Patients with Later Progression of rrDLBCL

Clinicopathologic Features	DLBCL (n=46)	rrDLBCL (n=18)	p
Age			0.417
<60	26	8	
≥60	20	10	
Gender			1
Male	23	9	
Female	23	9	
COO classification			0.24
GCB	10	1	
Non-GCB	36	17	
Ann Arbor Staging			0.044
I–II	19	2	
III–IV	27	16	
LDH level (mean±SD)	378.46±480.99	334.72±128.67	0.706

(*Continued*)

Table 1 (Continued).

Clinicopathologic Features	DLBCL (n=46)	rrDLBCL (n=18)	p
IPI			0.021
0–1	21	2	
2–3	19	10	
4–5	6	6	
ALB (mean±SD)	41.22±5.43	39.73±5.35	0.327
β-macroglobulin (mean±SD)	2.98±2.13	3.35±1.24	0.486
HGB (mean±SD)	123.02±16.27	117.22±19.07	0.227
ESR (mean±SD)	22.76+19.56	26.06+17.29	0.534
Ca+ (mean±SD)	2.33±0.17	2.27+0.24	0.199
DPL			0.268
Yes	19	4	
No	23	12	

Abbreviations: IPI, international prognostic index; LDH, lactate dehydrogenase; ALB, albumin; HGB, hemoglobin; ESR, erythrocyte sedimentation rate; DPL, double-protein-expression lymphomas; SD, standard deviation.

(Figure 2B–D), with a median follow-up time of (22.3 ± 2.2) months. These results indicated that *CD58* mutation might be an effective predictor of relapse or refractory status in primary DLBCL patients at the time of diagnosis.

Value of the Primary Mutation Profiles in Predicting the Curative Effect of DLBCL Patients in Response to Immunochemotherapy

In addition, we assessed the predictive value of the primary mutation profiles for determining the curative effect of immunochemotherapy in DLBCL patients. All patients in non-response group were diagnosed at stage III–IV, a significantly higher stage compared with the response group (p=0.008) (Table 2). In addition, the proportion of high-risk patients, as determined by the IPI, was significantly higher in the non-response group as compared with the response group (p=0.002) (Table 2). The mutation profiles showed that the frequency of *CARD11* mutations was significantly higher in non-response group as compared with the response group (Figures 3 and 4A). Multivariate analysis identified high-risk IPI (scores 4–5) (p=0.017) and CARD11 mutation (p=0.030) as two independent variables influencing the response of patients to immunochemotherapy (Figure 4B).

Longitudinal Monitoring of the Mutation Profiles of DLBCL Relapse

Luckily, primary and recurrent samples from two DLBCL patients were included in this study. To longitudinally monitor the mutation profiles of DLBCL relapse, we assessed the changes in Variant Allele Frequency (VAF) in the 2 cases. In case 1, the VAFs of *PRDM1* and *CD79B* increased in relapse sample as compared with the primary sample (Figure 4C). In case 2, the VAFs of *DUSP2* (dual specificity phosphatase 2) (NM_004418.3:exon3:c.719T>C:p.Ile240Thr), *STAT3* and *BTG2* increased in the relapse sample, while the VAFs of *SGK1* and another *DUSP2* variant (NM_004418.3:exon3:c.730+1G>C) decreased (Figure 4D). These results suggested that mutations with varying VAFs between primary and relapse conditions might contribute to the progression of DLBCL.

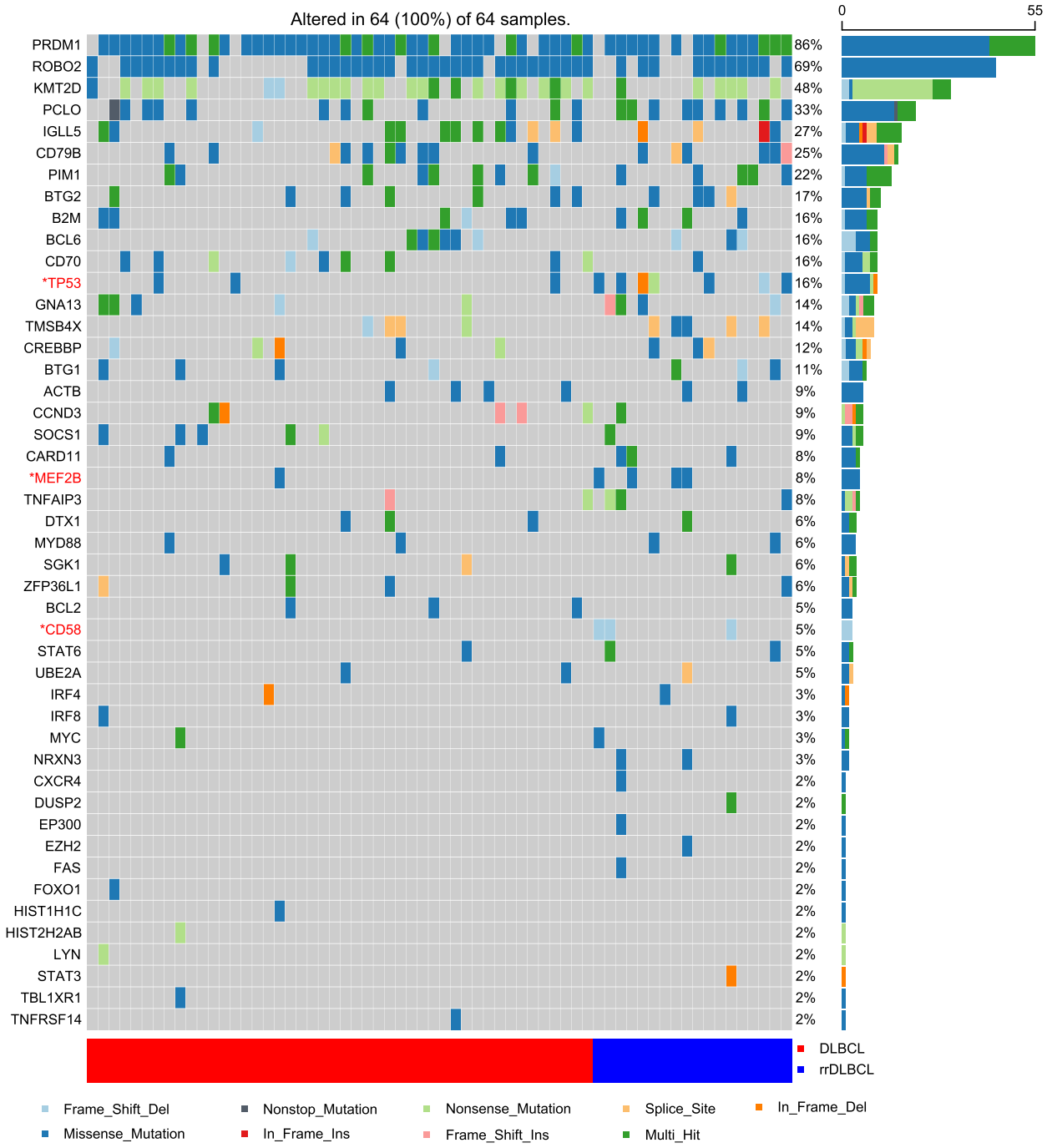

Figure 1 Comparison of mutation profiles in primary patients with and without progression to rrDLBCL. The horizontal histogram showed the mutation genes in the lymph node samples from primary patients who either progressed to or did not progress to rrDLBCL. * p value < 0.05, indicating the genes for which mutation frequency significantly differed between these two groups.

Horizontal Monitoring of Mutation Profiles in rrDLBCL

Also, we compared the mutation profiles of the biopsies from primary DLBCL patients and rrDLBCL patients. As shown in Figure 5, mutations in 7 genes (*HIST2H2AB, BCL2, NRXN3, FOXO1, HIST1H1C, LYN, TBL1XR1*) were detected exclusively in the primary DLBCL patients, while *EBF1* mutations was found only in the rrDLBCL biopsies. In addition, the frequency of *KMT2D* mutations was significantly lower in the rrDLBCL group (5/32) as compared with the primary

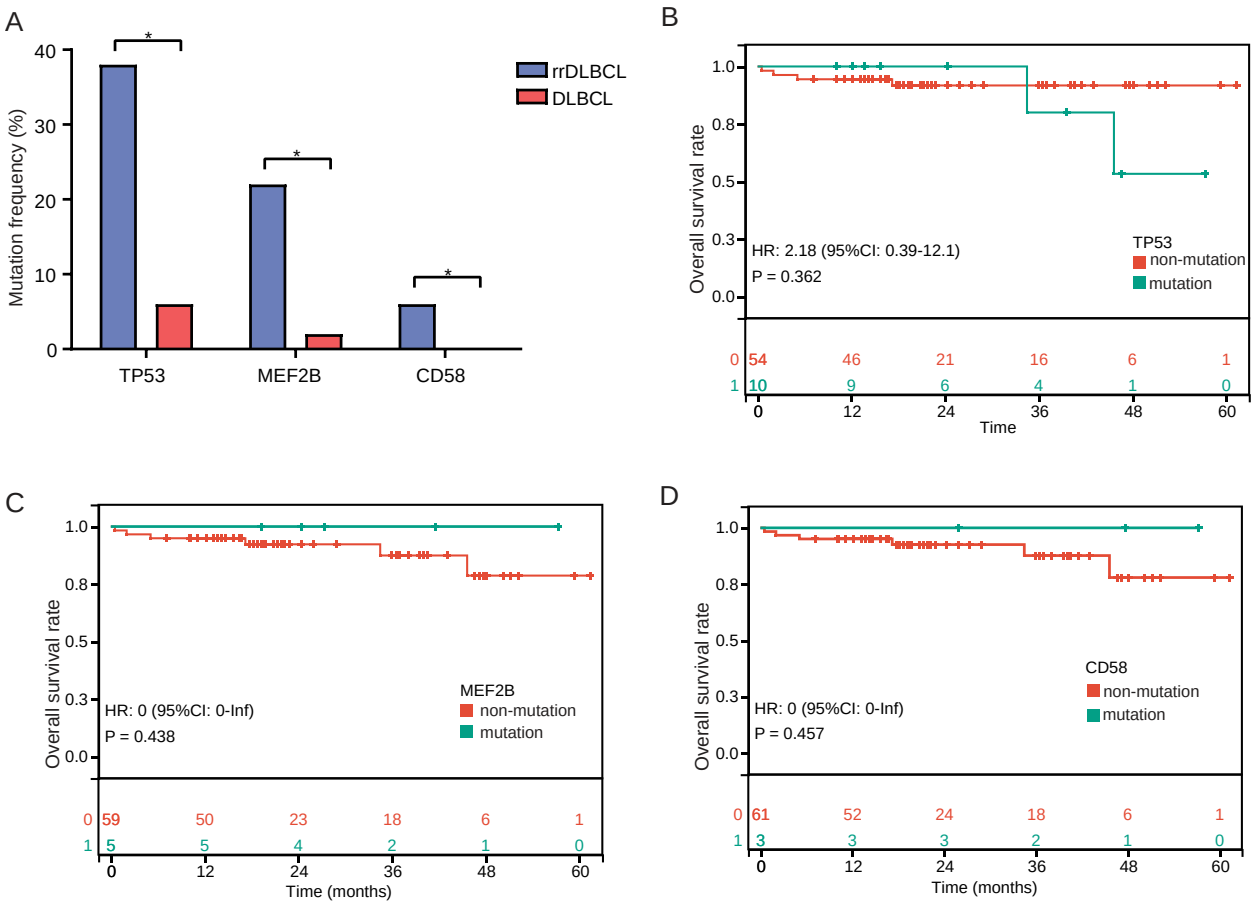

Figure 2 *TP53, MEF2B* and *CD58* mutations and their prognostic implications in DLBCL. (**A**) Mutation frequencies of *TP53, MEF2B* and *CD58* in diagnostic biopsies from DLBCL patients who proceeded to rrDLBCL versus those who did not (DLBCL group). (**B–D**) K-M curves depicting the relationship between the *TP53, MEF2B* and *CD58* mutations and the overall survival in DLBCL patients.

DLBCL group (31/64). These results demonstrated that mutations in *HIST2H2AB, BCL2, NRXN3, FOXO1, HIST1H1C, LYN, TBL1XR1, EBF1*, and *KMT2D* might play roles in the progression of rrDLBCL.

Discussion

In this study, we evaluated the predictive value of mutation landscapes in predicting the relapse/refractory progression in DLBCL. From the targeted sequencing of 55 genes, we found that the mutation frequencies of *TP53, MEF2B* and *CD58* were significantly increased in the diagnostic biopsies of patients who later developed relapse or refractory disease. This

Table 2 Clinicopathologic Features of Primary DLBCL Patients with Later Response to Immunochemotherapy

Clinicopathologic Features	Response Group (n=50)	Non-response Group (n=14)	p
Age			0.546
<60	28	6	
≥60	22	8	

(*Continued*)

Table 2 (Continued).

Clinicopathologic Features	Response Group (n=50)	Non-response Group (n=14)	p
Gender			0.762
Male	24	8	
Female	26	6	
COO classification			1
0	9	2	
1	41	12	
Ann Arbor Stage			0.008
I–II	21	0	
III–IV	29	14	
LDH (mean±SD)	365.72±462.78	367.71±130.89	0.987
IPI			0.002
0–3	45	7	
4–5	5	7	
ALB (mean±SD)	41.26±5.41	39.16±5.28	0.203
β-microglobulin (mean±SD)	2.84±2.02	3.94±1.20	0.058
HGB (mean±SD)	123.66±16.74	113.29±16.68	0.044
ESR (mean±SD	22.42±19.20	28.21±17.54	0.314
Ca2+ (mean±SD)	2.30±0.18	2.37±0.24	0.265
DPL			0.404
No	20	3	
Yes	26	9	

Abbreviations: IPI, international prognostic index; LDH, lactate dehydrogenase; ALB, albumin; HGB, hemoglobin; ESR, erythrocyte sedimentation rate; DPL, double-protein-expression lymphomas; SD, standard deviation.

finding is consistent with previous research that also highlighted the importance of specific genetic alterations in the progression of DLBCL.[15,16] Mutations in *TP53*, a well-established anti-tumor and therapeutic resistant gene, have been reported in 20–25% of DLBCL cases, with a similar incidence in both GCB and ABC subtypes.[9,11,17] Also, it has been demonstrated that *TP53* genes are more prevalent in rrDLBCL as compared with the primary DLBCL cases.[18] In addition, accumulating evidence has demonstrated that *TP53* mutation serves as a negative prognostic factor in DLBCL.[17,19,20] Targeting *TP53* mutation might represent a promising strategy for DLBCL patients harboring these mutations. Just so, several agents have been developed to reactivate functions of normal *TP53* in *TP53*-mutated tumor. These include molecules targeting a broad class of mutants to restore tumor suppressive functions (such as PRIMA, RITA, and scFv),[21–23] and compounds specifically targeting missense mutations (like Phikan059 targeting R220C).[24] *MEF2B* (Myocyte enhancer-binding factor 2B) is an independent regulator of *BCL6* expression, a crucial master regulator in germinal center formation.[25,26] *MEF2B* plays a significant role in regulating the proliferation of GC-derived lymphoma cells through partially modulating BCL6 expression.[27] Notably, we found that CD58 mutation was

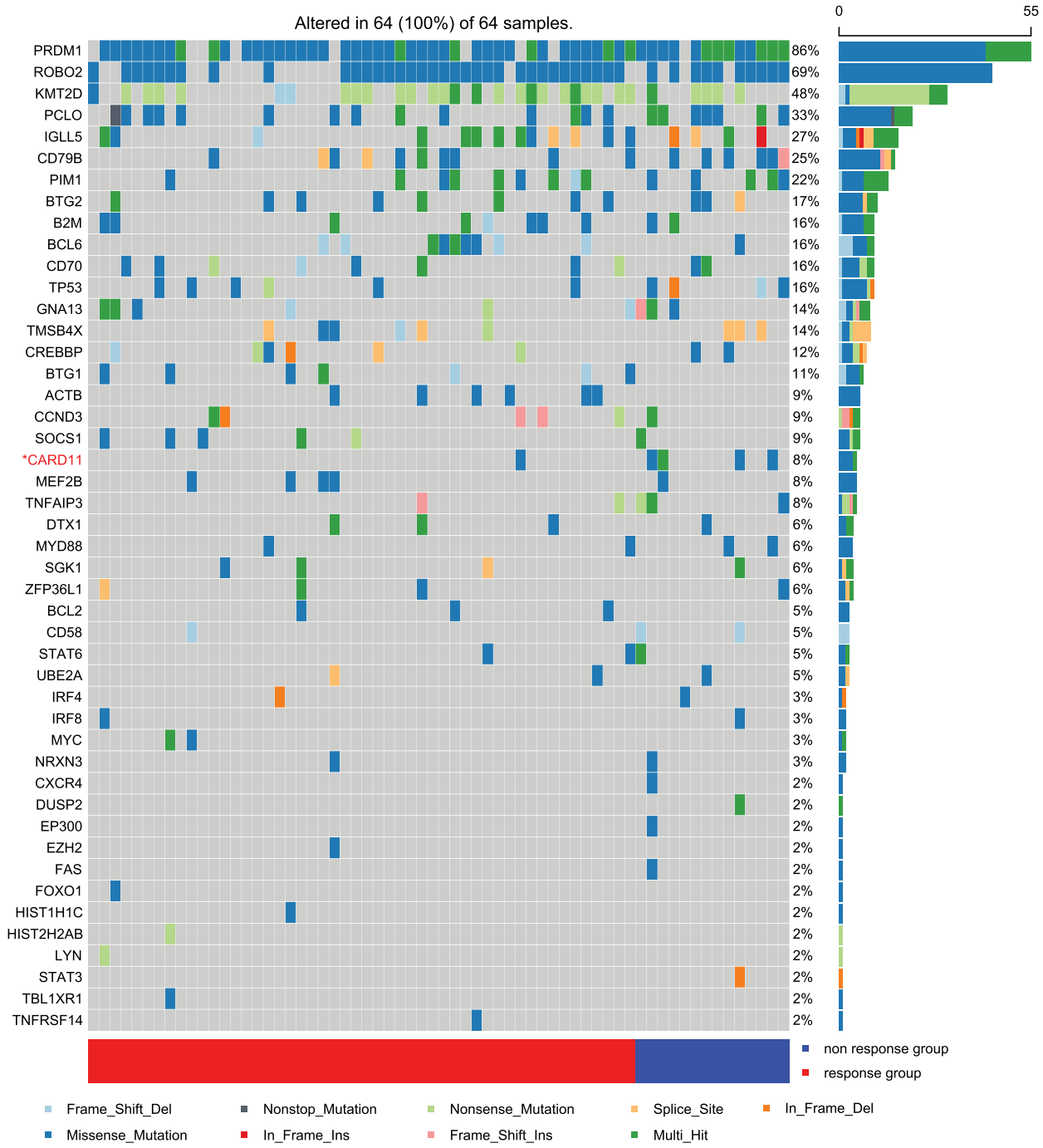

Figure 3 Comparison of mutation profiles in primary DLBCL patients based on response to immunochemotherapy. The horizontal histogram showed the mutation genes in the lymph node samples from primary patients who responded to the immunochemotherapy versus those who did not. * p value < 0.05 indicates genes for which mutation frequency significantly differed between responders and non-responders.

exclusively detected in the rrDLBCL cohort compared to the DLBCL cohort. This finding aligns with previous research that reported a higher mutation frequency of CD58 in rrDLBCL patients,[28] highlighting its potential as a potent marker for predicting relapse/refractory outcomes in primary DLBCL patients.[16] *CD58*-coded protein is a member of the immunoglobulin superfamily, which is vital for tumor recognition through binding to *CD2* expressed on T and NK cells.[29] Loss of *CD58* expression has been identified to be associated with worse overall and event-free survival of

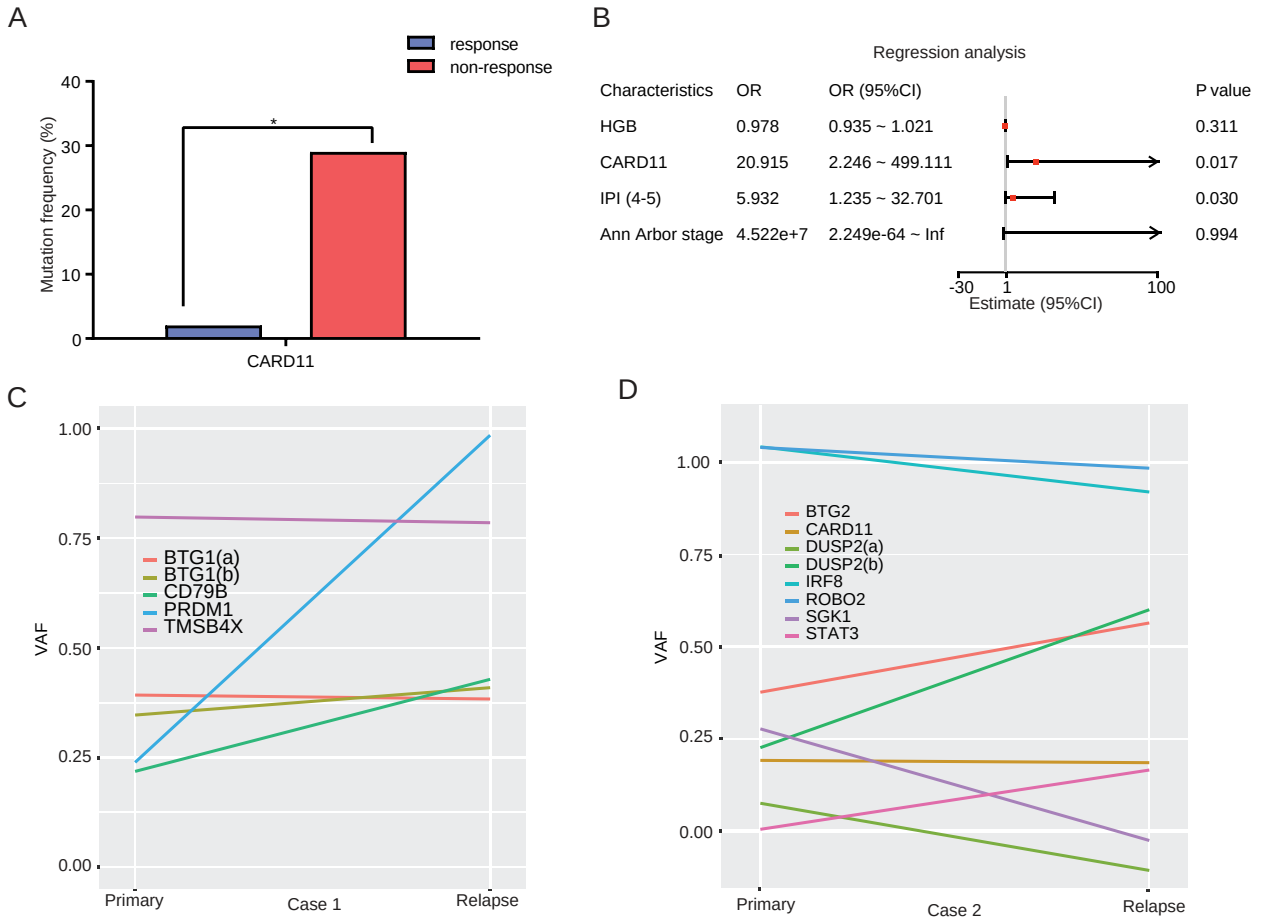

Figure 4 *CARD11* mutation status and Its Association with Immunochemotherapy Response and clonal evolution in DLBCL patients. (**A**) *CARD11* mutation frequencies in the diagnostic biopsies of DLBCL patients who responded to immunochemotherapy (response group) versus those who did not (non-response group). (**B**) Forest plot showing multivariate logistic analysis results to assess factors influencing the immunochemotherapy response in DLBCL patients. (**C** and **D**) Changes in the VAFs of mutated genes between the paired primary and relapsed DLBCL biopsies.

DLBCL patients.[30] Thus, we conjecture that restoring *CD58* expression may facilitate T and NK cell-mediated immune recognition to lymphoma cells, potentially increasing the efficacy of immunotherapy.

To further explore the value of mutations in predicting the relapse/refractory DLBCL, the primary DLBCL patients were divided into response group and non-response group based on the curative response to immunochemotherapy. Then, we compared the mutation profiles of the two groups. The results showed that the frequency of *CARD11* mutation was significantly higher in the non-response group as compared with the response group. This suggests that CARD11 mutations may be associated with patients' response to immunochemotherapy, echoing the findings of studies exploring molecular mechanisms underlying lymphoma cell sensitivity to treatment, possibly focusing on epigenetics or the immune microenvironment.[15] Also, multivariate logistic analysis showed that high-risk IPI (scores 4–5) and CARD11 mutation were the two independent variables influencing patient response to immunochemotherapy. *CARD11* encodes a multi-adaptor or immune signaling protein essential for propagating signals in immune cells. Mutations in *CARD11* have been implicated in carcinogenesis. Gain-of-function variants act downstream of T- and B-cell receptors in lymphoid cells, leading to NF-κB activation and promoting lymphogenesis.[31] On the contrary, loss-of-function variants are linked to severe combined immunodeficiency (SCID)[32] and combined immune deficiency.[33] In the current study, we found 6 missense variants of *CARD11* in 5 out of 64 cases with primary DLBCL. Previously, Lenz et al[34] detected the missense mutations of *CARD11* in 7 out of 73 ABC DLBCL biopsies, all within exons encoding the coiled-coil domain. Introducing *CARD11* coiled-coil domain mutations into lymphoma cells triggered constitutive NF-κB activation.[34]

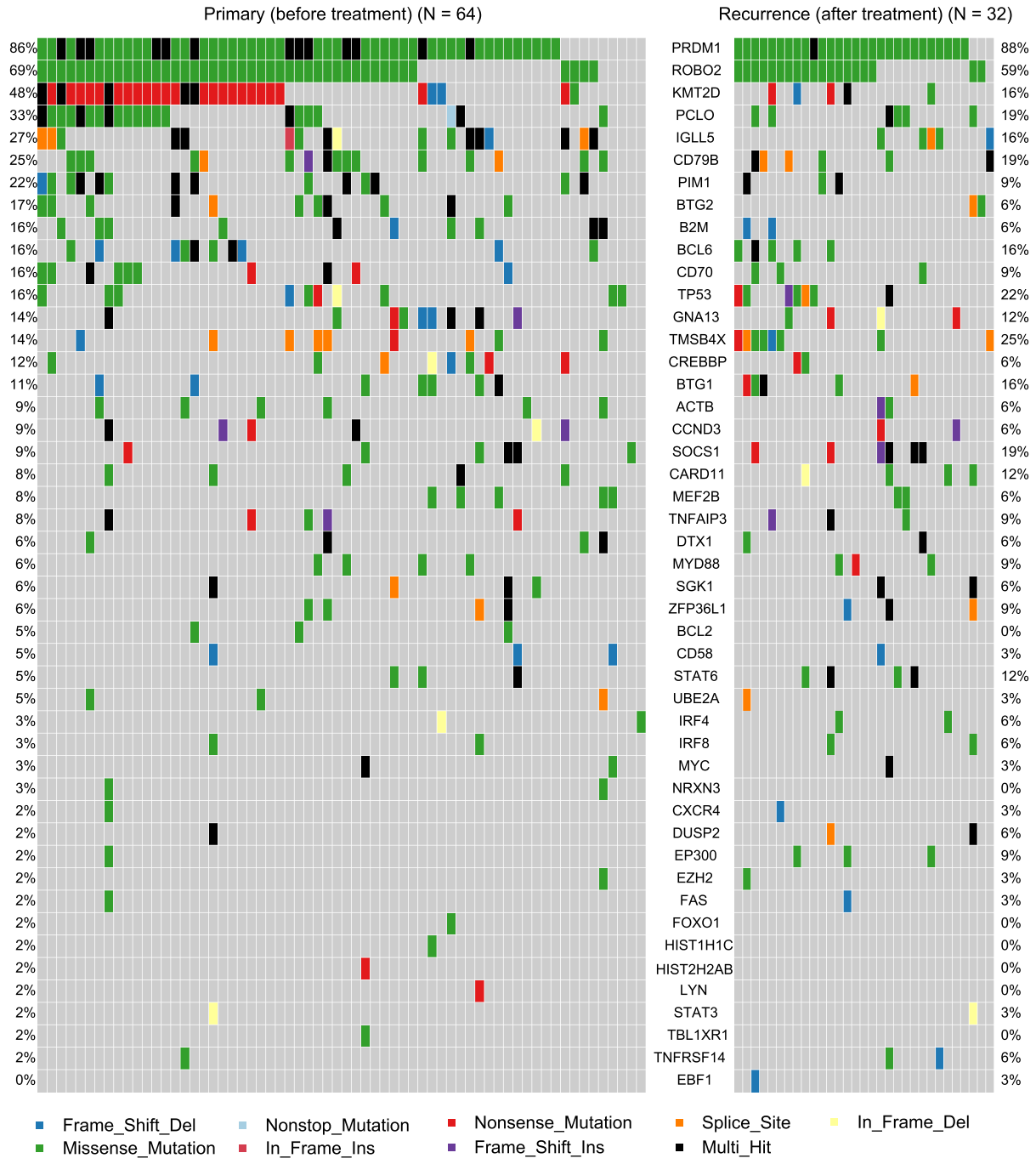

Figure 5 Mutation profiles in primary (pre-treatment) and recurrent (post-treatment) DLBCL patients. The horizontal histogram illustrates the differences in mutation profiles of lymph node samples between primary DLBCL and rrDLBCL patients.

Here, we found 4 *CARD11* variants located in exons encoding the coiled-coil domain and two in the linker domain. We infer the *CARD11* missense mutations may contribute to the resistance to immunochemotherapy through activating NF-κB signaling pathway.

In addition, we longitudinally evaluated the mutation profiles of DLBCL relapse in primary and relapse samples from two DLBCL patients. The integrated analysis showed that the VAFs of *PRDM1, CD79B, DUSP2* (NM_004418.3: exon3: c.719T>C: p.Ile240Thr), *STAT3* and *BTG2* mutants increased in relapse samples, while the VAFs of *SGK1* and *DUSP2*

(NM_004418.3:exon3:c.730+1G>C) decreased as compared with the diagnostic biopsies. *CD79B* was widely expressed on the tumor cells across various lymphomas regardless of stage, subtype, and cytogenetic and molecular features, suggesting that *CD79B* might serve as a potent target for CAR T-cell therapy of B-cell lymphomas.[35] Furthermore, we found variations in 7 genes (*HIST2H2AB, BCL2, NRXN3, FOXO1, HIST1H1C, LYN, TBL1XR1*) that were exclusively detected in primary DLBCL patients, while *EBF1* mutation was only detected in the rrDLBCL biopsies though horizontal monitoring of the mutation profiles in rrDLBCL. Using the Gene Expression Profiling Interactive Analysis database (GEPIA, http://gepia.cancer-pku.cn/), we found that the expression of *BCL2, FOXP1, TBL1XR1* and *EBF1* was significantly increased in DLBCL tissues as compared with normal tissues. Evidence has shown that three of these genes (*BCL2, FOXO1, TBL1XR1*) are involved in the NF-κB pathway,[36–38] and *EBF1* variants are common in DLBCL non-responders.[38]

In this study, several limitations should be acknowledged. Firstly, the small sample size may affect the generalizability of the findings. Secondly, we did not conduct a comprehensive analysis of the gene expression levels associated with significantly different mutation frequencies. Therefore, further research is needed to verify our results.

Collectively, this study enhances our understanding of the mechanisms driving the progression of rrDLBCL and suggests that *CD58* mutation might serve as a valuable marker to predict the relapse/refractory outcomes in primary DLBCL patients.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committee of the Peking University Third Hospital. The patients/participants provided their written informed consent to participate in this study. Registry and the Registration No: N/A. Animal Studies: N/A.

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Disclosure

The authors declare no conflicts of interest in this study.

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REVIEW

Management of Advanced Systemic Mastocytosis: Clinical Challenges

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Abstract: Advanced systemic mastocytosis (AdvSM) is a rare hematologic malignancy with organ damage and compromised life expectancy arising from organ accumulation of neoplastic mast cells. Identification of the gain-of-function *KIT*D816V in the majority of cases has accelerated pharmaceutical development culminating with the development of selective KIT inhibitors such as avapritinib. While the advent of these therapies has improved the quality and quantity of life in patients with AdvSM, current challenges remain in the management of this disease. In this review, we summarize the present and future therapeutics landscape of AdvSM, highlighting the development of novel KIT inhibitors including elenestinib and bezuclastinib. We also explore the continued role of additional treatment modalities including allogeneic stem cell transplantation before discussing unresolved clinical challenges in the management of AdvSM.

Keywords: systemic mastocytosis, KITD816V, avapritinib, midostaurin

Introduction

Systemic mastocytosis (SM) encompasses a diverse group of diseases involving neoplastic mast cells (MCs) that span a disease spectrum from indolent SM (ISM), which also includes a smoldering form (SSM), to an advanced form (AdvSM) that compromises life span. AdvSM is comprised of aggressive SM (ASM), SM with associated hematologic neoplasm (SM-AHN) and mast cell leukemia (MCL). The incidence of approximately 1.5 cases per 100,000 and a prevalence of 25 cases per 100,000 population.[1]

SM originates from clonal MCs derived from hematopoietic stem cells[2] that are inappropriately activated and accumulate in extramedullary tissues. These MCs can be associated with significant symptom burden as well as the potential to induce organomegaly with frank organ dysfunction. Both chronic and episodic activation of MCs results in varied symptomatology that is attributed to release of mast cell mediators such as histamine, heparin, leukotrienes, prostaglandins, platelet-activating factor, and proteases.[3]

The molecular pathogenesis of SM is characterized in the majority of patients by a gain of function oncogenic mutation in the stem cell factor (SCF) transmembrane class III receptor KIT (CD117).[4] KIT signaling promotes the proliferation, differentiation and activation of MCs. Over 95% of patients with SM harbor mutations in exon 17 of the *KIT* gene involving an adenine to thymine base switch at nucleotide position 2468, which results in an aspartic acid-to-valine change at codon 816 (D816V).[4,5] KIT mutations, as well as tryptase, can be effectively assayed in the peripheral blood. The functional consequence of this activation loop mutation is constitutive kinase activity leading to downstream signaling through MAPK, AKT, PI3K, and STAT signaling cascades.[6,7] It is now recognized that alternative activating mutations of *KIT* outside of D816V can also lead to MC activation, differentiation and proliferation and may alter the activation of the complex downstream signaling pathways.

While clinical manifestations in patients with ISM or SMM are primarily related to mediator symptoms, patients with AdvSM largely suffer from symptoms that are a result of MC proliferation leading to organomegaly (B-findings) or organ failure (C-findings).[8] ASM is defined by the presence of at least one C-findings, which are listed in Table 1, however patients with SM-AHN or MCL frequently also exhibit C-findings. Cytopenias are secondary to MC marrow infiltration

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Table 1 C Findings for the Diagnosis of Aggressive Systemic Mastocytosis

Cytopenias	ANC < 1 x 10^9/L Hgb < 10 g/dL Platelet < 100 x 10^9/L
Hepatopathy	Ascites and elevated liver enzymes* ± hepatomegaly or cirrhotic liver ± portal hypertension
Spleen	Palpable splenomegaly with hypersplenism ± weight loss ± hypoalbuminemia
GI tract	Malabsorption with hypoalbuminemia ± weight loss
Bone	Large-sized osteolysis (≥2 cm) with pathologic fracture ± bone pain

Notes: *Alkaline phosphatase levels are typically elevated in patients with advanced SM and SM-induced liver damage. In some of these patients, only elevated liver enzymes but no (clinically relevant) ascites is found.

in ASM and MCL, however in patients with SM-AHN low blood counts may also be related to associated neoplasm. Other features such as hepatomegaly and splenomegaly are present in approximately 40% of AdvSM patients.[9] Measurement of MC burden outside of C-findings occurs through several mechanisms. The most direct is histopathologic evaluation of MC tissue infiltration in the bone marrow (or other organs).[10] Tryptase is a serine protease that is concentrated in MCs and is a marker of MC activation.[11] The *KIT*D816V mutation is present in the vast majority of AdvSM patients and its allele burden is significantly higher in patients with AdvSM as compared to patients with ISM or SSM.[12] The key diagnostic, clinical and prognostic distinctions between SM subtypes are detailed in Table 2.

Table 2 Subtypes of Systemic Mastocytosis

	Indolent SM	**Smoldering SM**	**Aggressive SM**	**SM-AHN**	**Mast cell leukemia**
Diagnostic criteria					
Fulfills SM diagnostic criteria	Yes	Yes	Yes	Yes	Yes
B findings	No	Yes	–	–	–
C findings	No	No	Yes	–	–
Concurrent hematologic malignancy	No	No	No	Yes Most frequently CMML, MDS, MPN	–
Features of MCL	No	No	No	No	Yes ≥20% atypical immature mast cells in aspirate smear
Key clinical features					
	Prominent mediator symptoms: flushing, pruritus, nausea/ vomiting, abdominal pain, palpitations, headache, neuropsychiatric symptoms, anaphylaxis	Mediator symptoms, plus palpable hepatosplenomegaly	Mediator symptoms, B symptoms possible plus pathologic fractures, cytopenias, functional liver impairments and malabsorption	Mediator symptoms less likely, organomegaly cooccurring with manifestations of associated hematologic neoplasms	Extreme weight loss, fatigue, cytopenias, peptic ulcer disease coagulation

(*Continued*)

Table 2 (Continued).

	Indolent SM	Smoldering SM	Aggressive SM	SM-AHN	Mast cell leukemia
Prognosis					
Median OS	198 months (not significantly different than age and sex matched controls)[9]	52 months[15]	41 months[9]	24 months (but depends on the associated AHN)[9]	2 months[9]

These markers of MC burden and activation can also be used to assess response to treatment. In the International Working Group-Myeloproliferative Neoplasms Research and Treatment and European Competence Network on Mastocytosis (IWG-MRT-ECNM) response criteria, reduction in serum tryptase level to less than 20 ng/mL is required to fulfill a complete response (Table 3).[13] In addition, reduction in *KIT*D816V allele burden of at least 25% has been shown to be predictive of improved overall survival (OS).[14] In recent years, there has been significant progress in attaining these responses, and ultimately improving patient outcomes because of the development of selective *KIT*D816V inhibitors. Despite these improvements, several unresolved clinical challenges remain.

In this review, we describe the evolving risk stratification of AdvSM before detailing the current treatment landscape including midostaurin and avapritinib. We then describe novel therapeutics in development and then described remaining clinical challenges for the optimal management of AdvSM.

Table 3 IWG-MRT-ECNM Response Criteria for Advanced Systemic Mastocytosis

Complete remission (CR)*	
Requires all 4 criteria and response duration must be ≥ 12 wk	No presence of compact neoplastic mast cell aggregates in the BM or other biopsied extracutaneous organ
	Serum tryptase level < 20 ng/mL†
	Peripheral blood count remission defined as ANC ≥ 1×10^9/L with normal differential, Hb level ≥ 11 g/dL, and platelet count ≥ 100×10^9/L
	Complete resolution of palpable hepatosplenomegaly and all biopsy-proven or suspected SM-related organ damage (CI findings)‡
Partial remission (PR)*	
Requires all 3 criteria and response duration must be ≥ 12 weeks, in the absence of both CR and progressive disease (PD)	Reduction by ≥ 50% in neoplastic MCs in the marrow and/or or other extracutaneous organ at biopsy demonstrating eligible SM-related organ damage
	Reduction of serum tryptase level by ≥ 50%†
	Resolution of 1 or more biopsy-proven or suspected SM-related organ damage (CI finding(s))‡
Clinical improvement (CI)*	
Response duration must be ≥ 12 weeks	Requires 1 or more of the nonhematologic and/or hematologic response criteria to be in the absence of both CR/PR
	assignment or progressive disease (PD)
Stable disease (SD)	
	Not meeting criteria for CR, PR, CI, or PD

(*Continued*)

Table 3 (Continued).

Progressive disease (PD)[§]	
Requires at least 1 element of either criteria 1 or 2 and duration must be ≥ 8 weeks	(1) For patients with baseline grade 2 nonhematologic organ damage: a) worsening by 1 grade, AND b) minimum 100% increase (doubling) of laboratory abnormality. For patients with baseline ≥ grade 2 albumin: (a) worsening by 1 grade, AND (b) decrease by ≥ 0.5 g/dL. For patients with baseline ≥ grade 3 nonhematologic organ damage: minimum 100% increase (doubling) of laboratory abnormality. For patients with baseline ≥ grade 2 transfusion-independent anemia or thrombocytopenia: New transfusion dependence of ≥ 4 units of RBCs or platelets at 8 wk. For patients with baseline transfusion-dependent anemia or thrombocytopenia: ≥100% increase in the average transfusion frequency for an 8-wk period compared with the 12-wk pretreatment period For patients with baseline grade ≥ grade 3 neutropenia: (a) > 50% decrease in neutrophil count, AND (b) absolute decrease of neutrophil count of ≥ 250/mm^3, AND c) grade 4 (2) Development of at least 10-cm palpable symptomatic splenomegaly for a baseline spleen size of not palpable or ≤ 5 cm, OR if baseline symptomatic splenomegaly is > 5 cm, a > 50% worsening and development of at least 10 cm of palpable symptomatic splenomegaly compared with the baseline value.[¶]
Loss of response (LOR)	
	Loss of a documented CR, PR, or CI that must be for ≥8 wk. Downgrading of CR to PR or PR to CI is considered as such but is not considered as loss of response unless CI is also lost for a minimum of 8 wk. The baseline value for LOR is the pretreatment measurement(s) and not the nadir values during response.

Notes: Guidelines for adjudicating response are as follows: (1) Only disease-related ≥ grade 2 organ damage is evaluable as a primary endpoint in clinical trials. (2) Response adjudications of CR, PR, SD, PD, and LOR should only be applied to these ≥ grade 2 organ damage findings in the context of trials. (3) Disease status at the time of patient removal from the study singularly relates to the updated status of initial ≥ grade 2 organ damage finding(s). (4) Exclusion of drug-related toxicity and/or other clinical issues (eg, gastrointestinal tract bleeding in the case of worsening anemia/transfusion-dependence) should be undertaken before assigning the designation PD or LOR in a patient with worsening of baseline ≥ grade 2 organ damage. *Responses that are not maintained or confirmed for a period of at least 12 wk do not fulfill criteria for CR, PR, or CI; however, both maintained and unmaintained (< 12-wk duration) responses in organ damage should be recorded to determine median duration of response. [†]Only valid as a response criterion if the pretreatment serum tryptase level is ≥ 40 ng/mL. [‡]Biopsy of organ(s) in addition to the BM to evaluate for SM-related organ damage may be considered. [§]Preservation of at least one CI finding permits a patient to maintain the response of "CI" if 1 or more CI findings are lost but none meet criteria for progressive disease (PD). However, if 1 or more of the CI findings become PD, then the CI finding assignment is lost and the patient meets criteria for PD. The baseline value for evaluating PD is the pretreatment measurement(s). The PD findings must be considered related to the underlying disease and not to other clinical factors. Progression of an underlying chronic myeloid neoplasm to AML is also considered PD in the setting of clinical trials. [¶]For clinical trials using 3D computed tomography or magnetic resonance imaging as an additional modality to quantify organomegaly, progression in splenomegaly is defined as an increase in spleen volume of at least 25%. Adapted from reference.[13]

Risk Stratification

In 2022, both the WHO and the International Consensus Classification (ICC) introduced updates for the classification and diagnostic criteria of SM.[16,17] The ICC upheld the five subtypes of systemic mastocytosis from 2016, while the WHO 5th edition (2022) classification system categorizes SM into six different classes with one new variant, BMM. The first category is ISM which meets the criteria of SM, requiring at least 1 major and 1 minor or 3 major SM criteria, without additional B or C findings. This is the most common variant of SM and is associated with a favorable prognosis, both in terms of OS and leukemia-free survival. ISM progresses slowly and OS that is likely similar to that of the age- and sex-matched population based off a retrospective study with 342 patients.[9] However, a subsequent analysis of OS in the ISM population demonstrated a modest but statistically significant decrease in survival for patients in the first 4 years of their disease course, regardless of whether their disease progressed to advanced SM.[18] Patients with indolent or smoldering

SM tend to be younger at presentation, present with a higher percentage of skin lesions and GI symptoms and are less likely to exhibit constitutional symptoms or hepatosplenomegaly.

The WHO 5th edition (2022) added an additional variant of SM not included in the 2016 classification system or by the ICC. This additional category, bone marrow mastocytosis (BMM) is defined as neoplastic MC proliferation solely involving the BM. BMM is characterized by limited BM infiltration, absence of cutaneous lesions, normal or minimally elevated serum tryptase levels (<125 ng/mL), older age and male predominance.[19] Patients with isolated bone marrow neoplastic mast cell involvement but who also have B findings or a tryptase level ≥125 ng/mL have an inferior PFS and OS as compared to BMM or ISM patients without these two clinical features.[20]

SSM is also a recognized diagnostic category. This is considered an intermediate-stage variant and is characterized by 2 or more "B findings". The prognosis is worse compared to ISM but not as aggressive as the AdvSM categories. Patients with SSM tend to present at an older age, have higher bone marrow MC burden, higher serum tryptase level, as well as increased prevalence of palpable hepatosplenomegaly. SSM is associated with inferior OS and an increased risk of progression to ASM compared to the other ISM subtypes.[21]

Aggressive Mastocytosis (ASM) is characterized by the presence of "C findings". It requires fulfilling the SM criteria with the presence of ≥1 C finding, which include impairment or loss of organ function due to mast cell infiltrates (Table 1).[22] Associated symptoms include constitutional symptoms, hepatosplenomegaly (with impairment of liver function, ascites or portal hypertension), lymphadenopathy, severe anemia (hemoglobin <10g/dL) and/or thrombocytopenia (platelets 100×10^9/L), leukocytosis (ANC < $1.0x10^9$/L due to bone marrow dysfunction). Due to gastrointestinal mast cell infiltrates, patients may have abdominal pain, nausea, vomiting, diarrhea or GI bleeding and may have malabsorption with hypoalbuminemia and weight loss. They may have musculoskeletal pain or osteopenia, due to skeletal involvement, which may manifest with osteoporosis and pathologic fractures.[23,24]

The next category is SM with associated hematological neoplasm (SM-AHN), which requires meeting criteria for both SM and another hematologic malignancy. SM-AHN has a more aggressive clinical course. The AHN component most often includes chronic myelomonocytic leukemia (CMML), MDS, myeloproliferative neoplasms (MPN), AML, B-cell lymphoma and plasma cell neoplasms.[25] SM-AHN is associated with an inferior OS, however the prognosis is generally determined by the aggressiveness of the AHN.[26]

Mast cell leukemia (MCL) is the rarest subtype, extremely aggressive and categorized by the highest mortality.[27] This subtype accounts for less than 1% of all SM cases. MCL is considered a form of acute leukemia and is defined by the presence of at least 20% neoplastic immature MCs in the bone marrow and 10% in the PB. MCL can either be secondary MCL following progression from another SM or can present as primary MCL.[28]

Associated Mutations

More than 90% of typical ISM and 70% of AdvSM carry acquired point mutation in the *KIT* gene. Additional somatic mutations (*ASXL1, RUNX1, SRSF2, NRAS)* have been found in 90% of ASM patients.[29] A recent next-generation sequencing study of one hundred and fifty patients revealed 75% of patients possessed *KITD816V.* Sixty-three (42%) patients were either unmutated or had no additional mutation other than *KIT*D816V. For the remaining 87 patients, a total of 148 non-KIT mutations were identified: 46 (31%) patients harbored one mutation, 24 (16%) two mutations, 14 (9%) three mutations and three (2%) had four mutations. The most frequently mutated non-KIT genes were *TET2 (29%), ASXL1 (17%), CBL (11%), SF3B1/DNMT3A/JAK2 (6% each), U2AF1 (4%)*, and *RUNX1 (3%). ASXL1* and *RUNX1* mutations are associated with inferior survival, independent of age and WHO subtype.[30] The frequency of these mutations is significantly greater in AdvSM as compared to non-AdvSM, 19 of 27 non-KIT mutations were found in patients with advanced SM.[23]

Prognostic Scoring Systems

There are several prognostic scoring systems to help categorize AdvSM based on clinical and molecular factors. However, these are often challenging to incorporate into clinical practice.

The Mayo Alliance Prognostic System (MAPS) was developed in 2018 and incorporates two different models. The scoring system was developed based on 580 patients seen at Mayo Clinic between 1968 and 2015. The first system includes

only 5 clinical variables, which were advanced SM vs ISM/SSM, age >60 years, platelets <150x10^9/L, anemia below sex-adjusted normal and serum ALP above normal range. Survival was directly correlated with the number of risk factors, with a great prognosis for patient with ≤1 risk factor (median survival not reached) and poor outcomes for patients 4 or 5 risk factors (median survival, 9–27 months). This study showed that the model was equally effective whether it was applied to patients with AdvSM or ISM/SSM. The second prognostic model incorporates adverse molecular data, such as the presence of *ASXL1/RUNX1/NRAS* and incorporated the previously defined clinical variables. The OS without adverse mutations was median 70 months compared to 10 months with the identification of an adverse mutation present *(ASXL1, RUNX1, NRAS)*.[31] These models included the WHO classification system for SM, which is subject to variable interpretation. A subsequent WHO-independent MAPS system was developed to eliminate this subjectivity, which focused solely on age, platelet count, sex-adjusted hemoglobin, increased alkaline phosphatase and serum albumin.[31]

The Mayo clinic group subsequently proposed a Mutation-Augmented Prognostic Scoring System (MAPSS) with next-generation sequencing of 27 relevant genes in 150 SM patients that could be integrated into a prognostic model. In multivariate analysis, age >60 years, hemoglobin <10 g/dL or transfusion-dependence, platelet count <150x10^9/L, serum albumin <3.5 g/dL, and *ASXL1* mutation were associated with inferior survival. This study stratified ASM into three distinct risk groups: low-risk, intermediate-risk and high risk with associated median survivals of 86, 21, and 5 months, respectively.[30]

In 2019, the International Prognostic Scoring System of Mastocytosis (IPSM) was created based on a study by Sperr at all.[32] This study utilized a database of 1639 patients with SM and divided patients into three groups based on age >60 years and elevated alkaline phosphatase value: low (no risk factors), intermediate 1 (one risk factor) and intermediate 2 (two risk factors). In patients with AdvSM (n=259), age 60 years or older concentration of tryptase 125 ng/mL or higher leukocyte count of 16 x 10^9/L or higher, hemoglobin of ≤11 g/dL, platelet count of ≤100 x 10^9 /L, and skin involvement were independent prognostic factors for OS in multivariate analyses. Each risk factor with an HR greater than 1.50 scored 1 point and risk factors with an HR of 0.50 or lower scored −1 point. By adding the risk factors, four different risk groups were established. Based on these variables, a separate score was established with four risk categories for AdvSM. OS and PFS differed significantly among these groups ($p < 0.0001$).[33]

The Global Prognostic Score for Mastocytosis (GPSM) further identified variables that impacted disease progression (GPSM-PFS) and survival (GPSM-OS) and were based on platelet count ≤100 × 10^9 cells per L, serum β2-microglobulin ≥2.5 µg/mL, and serum baseline tryptase ≥125 µg/L for PFS and hemoglobin ≤11 g/dL, serum alkaline phosphatase ≥140 IU/L, and at least one mutation in *SRSF2, ASXL1, RUNX1*, or *DNMT3* for OS. The GPSM-PFS and GPSM-OS models were able to discriminate between low-risk and high-risk patients for worse PFS and OS in the discovery and validation cohorts, with a discovery cohort of 422 and an independent cohort of 853 patients, respectively. This prognostic tool was able to predict survival outcomes in patients with SM.[34]

The Mutation-Adjusted Risk Score (MARS) was developed from a study analyzing 383 patients with ASM from the German Registry on Disorders of Eosinophils and Mast cells. Multivariable analysis identified risk factors associated with OS: age >60 years, hemoglobin <10 g/dL, thrombocytopenia (<100 x 10^9/L) presence of one high molecular risk gene mutation (*SRSF2, ASXL1* and/or *RUNX1*) and presence of two or more high molecular risk gene mutations. This MARS was independent of WHO classification type and was confirmed with an independent validation cohort.[35]

One registry-based study reviewed 2607 patients enrolled within the European Competence Network on Mastocytosis (ECNM) and 575 patients enrolled within the German Registry on Eosinophils and Mast cells (GRM). This study found that many patients with AdvSM are misdiagnosed or experience delayed diagnosis especially if patients lack skin involvement or MC mediator-related symptoms during presentation. This study identified the following serum parameters as the most relevant: tryptase, alkaline phosphatase, B2-microglobulin, lactate dehydrogenase, albumin, vitamin B12 and C-reactive protein and concluded that serum chemistry profiling is crucial for diagnosis and prognostication.[36] A panel of experts from the ECNM together with an expert panel of the American Initiative in Mast Cell Diseases (AIM) reviewed these prognostic scoring systems and recommended utilizing the IPSM and GPSM-PFS for non-aggressive SM and the IPSM, GPSM and MARS for patients with ASM.[37]

Treatment

Supportive Care

Although more pronounced in patients with ISM, mediator symptoms can be present in AdvSM patients and frequently require therapies, which can ameliorate the effects of MC degranulation. These therapies including histamine blockers, leukotriene inhibitors, sodium cromolyn, proton pump inhibitors and corticosteroids should be tailored based on mediator symptoms. Avoidance of symptom triggers should be discussed with all patients and those at risk for anaphylaxis should carry a self-injected epinephrine kit (EpiPen) at all times.[38] In patients with AdvSM, additional supportive measures that may be required including screening and management of osteoporosis as well as transfusion support in addition to AdvSM directed therapy described below.

Midostaurin

Midostaurin is a multi-kinase inhibitor with activity against both wildtype and D816V mutated *KIT* that was approved for the treatment of AdvSM by the FDA in 2017.[39] This agent has been evaluated in an open-label Phase 2 study, which included 116 AdvSM patients, of which 89 were included in the primary efficacy analysis (16 ASM, 57 SM-AHN, and 16 MCL) who were treated with midostaurin at a dose of 100mg twice daily. The primary endpoint of overall response was 60% by modified Valent and Cheson criteria.[24,40] However, a post-hoc analysis using the IWG-MRT-ECNM consensus criteria identified the overall response rate (ORR) to be 28% when including clinical improvement (CI) as a response.[41] Breakdown of responses among subtype is shown in Table 4. Responses were durable with a median duration of 24.1 months and there were significant reductions in MC burden in the bone marrow as well as serum tryptase levels. The median OS was 28.7 months with median PFS of 14.1 months. Importantly, there was also reversal of organ damage as evidenced by normalization of hypoalbuminemia in 58% of patients, and achievement of red blood cell and platelet transfusion independence in 40% and 100% of dependent patients, respectively. However, gastrointestinal adverse events (AEs) were common, with all grade nausea being observed in 79% of patients, vomiting in 66% of patients, and in 54% of patients. Dose reductions because of AEs were required in 41% of patients and AEs led to discontinuation in 22% of patients.[42]

Subsequent studies have aimed to compare midostaurin with cladribine using propensity-score matching and demonstrated superior OS (4.2 years versus 1.9 years) and leukemia-free survival (2.7 years versus 1.3 years).[43] Predictors of superior OS in midostaurin-treated AdvSM patients include reduction of *KIT*D816V allele burden by ≥25%. Of note, the same analysis also demonstrated that clonal evolution occurs while receiving midostaurin treatment, with acquisition of new mutations in *KRAS, NRAS, RUNX1, IDH2*, and *NPM1*.[14] Midostaurin was the standard front-line therapy for AdvSM patients, however its use has largely been replaced by the introduction of avapritinib.

Avapritinib

Avapritinib is a highly selective type 1 inhibitor of *KIT*D816V with higher potency as compared with midostaurin (IC_{50} 0.27 versus 2.9) with negligible activity against wildtype KIT.[44] Avapritinib was evaluated in AdvSM patients in the

Table 4 Outcomes of Midostaurin and Avapritinib Clinical Trials in Advanced Systemic Mastocytosis

Agent	MoA	Study name	N	Response rates by IWG-MRT-ECNM				Other
				Overall	ASM	SM-AHN	MCL	
Midostaurin	Multikinase inhibitor	CPKC412D2201	113	28%	60%	21%	33%	
Avapritinib	Selective KIT inhibitor	EXPLORER Phase 1	53	75%	100%	76%	69%	ORR 59% with prior midostaurin exposure
		PATHFINDER Phase 2	32	75%	100%	81%	25%	

phase 1 EXPLORER study which enrolled 86 AdvSM patients. After dose escalation, the 200mg and 300mg dose cohorts were expanded. Among 69 evaluable patients, the ORR by IWG criteria was 75% (with breakdown by subtype shown in Table 3). ORR was higher in midostaurin naïve patients as compared to those previously treated (83% versus 59%). Importantly, 36% of patients experience a CR or CR with partial hematologic recovery (CRh) and 30% of patients experience of molecular CR.[45] Bone marrow evaluation demonstrated reduction of MC aggregates, loss of CD25 expression, improvement in bone marrow fibrosis and reversal of spindled MC morphology.[46] During a median follow-up of 23 months, 14 patients (20%) experienced disease progression, including 6 patients (9%) who developed acute myeloid leukemia (AML).[45]

Avapritinib has also been tested in the phase 2 PATHFINDER study at a dose of 200mg daily. The interim results have been published which includes 62 AdvSM patients with evaluable mIWG–MRT–ECNM C-finding or MCL. The ORR was also 75% including 19% with CR/CRh. Similar to EXPLORER, there was evidence of profound reduction in MC burden with 60% of patients attaining complete elimination of bone marrow MCs and 93% of patients attaining ≥50% reduction in tryptase levels. In addition, 60% of patients had ≥50% reduction in peripheral blood *KIT*D816V allele burden.[47] The results after three years of follow-up were recently presented and demonstrated that the median duration of response and OS was not reached.[48]

Despite these substantial benefits with avapritinib treatment in AdvSM, there are several safety considerations worth highlighting. In a pooled analysis of avapritinib 200mg daily from the EXPLORER and PATHFINDER trials, the most common non-hematologic AEs included peripheral/periorbital edema (all grades 81%), diarrhea (34%), nausea (31%), fatigue/asthenia (28%), and cognitive effects (25%). The cognitive effects included memory impairment and encephalopathy, which were reversible with dose reduction or interruption.[49] In addition, intracranial hemorrhage occurred in 9 patients (13%) of patients in the EXPLORER study, although it was asymptomatic in 5 patients and occurred in the setting of antecedent thrombocytopenia in 7 patients.[45] Based on these findings, a platelet count cut off of 50×10^9/L was added as an amendment in PATHFINDER. Only 1 patient (2%) experienced an intracranial hemorrhage before this exclusion criteria was implemented.[47] Hematologic toxicities with avapritinib include neutropenia, anemia, and thrombocytopenia which were grade 3 in 16%, 27% and 30% of patients, respectively.[45]

As avapritinib has only been evaluated in single-arm studies, a recent retrospective analysis attempted to assess the difference between avapritinib and best available therapy (BAT) after adjusting for key covariates. Comparing 176 avapritinib treated patients in EXPLORER and PATHFINDER to 141 patients treated with 222 lines of therapy, which included tyrosine kinase inhibitors, mostly midostaurin (51%), cytoreductive agents including cladribine (25%) and hydroxyurea (9%), there was an improved OS with a hazard ratio (HR) of 0.48 (p=0.004) and significantly longer duration of treatment (HR 0.36, p<0.001). Tryptase reduction was also significantly deeper in the avapritinib group as compared to BAT.[50] These results support the efficacy of avapritinib in patients with AdvSM in lieu of randomized controlled trial data.

Avapritinib is currently the standard therapy for newly diagnosed or previously treated AdvSM patients. Caution should be taken in patients with baseline thrombocytopenia, particularly those who have SM-AHN.

Other Therapies

Although largely supplanted by the availability of selective KIT inhibitors, therapies traditionally utilized for the treatment of AdvSM still may have a role. Cladribine, a nucleoside analogue, is an effective agent for rapid de-bulking of MCs or in AdvSM patients relapsed or refractory to other agents. Of note, while this agent is associated with clinical responses, treatment-related toxicity can also occur. This is highlighted in one of the largest experiences of cladribine in SM, a French nationwide retrospective experience which included 32 patients with AdvSM. The ORR was 50% in AdvSM patients with a duration of response of 2.5 years for ASM and 4.8 years in SM-AHN. Myelosuppression is relatively common with neutropenia in 47% of patients and 22% experiencing infectious complications in the total cohort (including ISM patients).[51]

Interferon alfa (IFN-a) treatment has also been historically used for the treatment of all subtypes of SM.[52] In a report of 36 AdvSM patients treatment with IFN-a with or without prednisone resulted in an ORR of 60% and 45% for ASM and SM-AHN, respectively.[53] Notable toxicities include depression, thrombocytopenia, and flu-like symptoms after

administration.[54] A pegylated version has less frequent dosing and improved tolerability. We reserve IFN-a for patients with slowly progressive AdvSM who are not candidates for other therapies. Hydroxyurea has also been explored, although there is minimal data to effectively characterize the clinical benefit.[53]

Finally, imatinib can be utilized in the rare patient who does not harbor *KIT*D816V mutation or who has a mutation outside of exon 17.[55] Imatinib has demonstrated efficacy against wild-type *KIT* and certain trans-membrane and juxta-membrane *KIT* mutants, however, the *KIT*D816V mutation is resistant to imatinib.[56]

Allogeneic Stem Cell Transplantation

Allogeneic stem cell transplantation (ASCT) is a potentially curative option in patients with AdvSM, although experience to date has largely been reported in SM-AHN. The largest retrospective series of 57 patients (38 with SM-AHN, 7 with ASM, 12 with MCL) demonstrated significant decreases in bone marrow MC percentages and serum tryptase levels. All patients with SM-AHN achieved a CR from their associated hematologic disease but 10 went on to relapse and five of those ultimately died. MCL patients had the highest rate of treatment-related mortality and highest primary resistance to ASCT. OS at 3 years was 43%, 17%, and 74% for ASM, MCL, SM-AHN, respectively.[57] Consensus opinion on the role of ASCT in AdvSM recommends for MC debulking with the use of a KIT inhibitor or chemotherapy before proceeding to ASCT, particularly in the setting of MCL. Outside of this subtype, appropriate AdvSM patients for ASCT include younger patients who have achieved a response and have a suitable donor.[58] However, the calculation of when to proceed to ASCT has been complicated by the availability of selective KIT inhibitors. In follow-up from the phase 1 avapritinib study, the 2-year OS rates were 100%, 92%, 67%, for ASM, MCL, SM-AHN, respectively, which compares favorably to ASCT data with the exception of SM-AHN.[45] Therefore, in the case of patients with SM-AHN, we preferentially triage patients to ASCT if eligible as a curative modality for both the SM and AHN components.

Novel Agents

The development of TKIs has revolutionized treatment for AdvSM. Previously, cytoreductive therapy was the mainstay of treatment and now selective KIT inhibitors such as avapritinib represent the standard of care he D816V *KIT* point mutation also confers resistance against several tyrosine kinase inhibitors including imatinib.[59] There are several clinical trials underway evaluating novel TKIs in this patient population.

Elenestinib (BLU-263)

Elenestinib (BLU-263) is a potent and selective small-molecule inhibitor of *KIT*D816V with limited central nervous system (CNS) penetration and daily dosing strategy. This agent showed favorable tolerability and safety profile in a phase 1 trial. The ongoing randomized double-blind phase 2/3 HARBOR trial (NCT04910685) includes patients with ISM. After 12 weeks of therapy, elenestinib demonstrated beneficial effects on total symptom score and biomarkers of MC burden. Patients receiving elenestinib at 25 mg, 50 mg, and 100 mg doses showed reduction from baseline for tryptase (−15.4%, −50.9%, and −68.4% vs 3.3 respectively) and *KIT*D816V VAF (−37.5%, −70.3%, and −77.0% vs −2.5%, respectively) as compared to placebo.[60] This agent is also being evaluated in the AZURE phase 1/2 trial (NCT05609942) for patients with advanced AdvSM as a monotherapy or in combination with azacytidine if indicated for an AHN.[61]

Bezuclastinib (CGT9486)

Bezuclastinib is a potent and selective inhibitor of *KIT*D816V, with minimal effects on other kinases. This agent has low CNS penetration, high selectivity, and favorable pharmacokinetics, which ideally minimize systemic and CNS side effects. Bezuclastinib is currently being evaluated in a Phase 2 clinical trial, APEX (NCT04996875) with 140 adult patients with AdvSM per WHO criteria with SM-related organ damage, baseline serum tryptase of ≥20 ng/mL and could have received prior TKI therapy.[62] As of April 2023, Part 1 was fully enrolled with 33 AdvSM patients. Data with 32 evaluable patients showed 56% ORR rate and 75% ORR as well as deep reductions across biomarkers of MC activity, with 94% of patients experiencing a ≥50% decrease in serum tryptase, 93% with ≥50% reduction in *KIT*D816V VAF and 97% of patients with a ≥50% bone marrow MC burden. The majority of AEs were low grade and reversible. The most

frequent AEs were hair color changes 34%, thrombocytopenia 22%, increases in transaminase 22%, neutropenia 19% and taste disorder 19% and no reported cognitive or bleeding events.[63,64]

Conclusions and Unresolved Clinical Challenges

There has been undeniable progress over the last decade in the treatment of AdvSM, culminating in the approval of the selective *KIT*D816V inhibitor avapritinib. However, there remain several unresolved clinical challenges. For one, with the potential introduction of additional selective *KIT* inhibitors including BLU-263 and bezuclastinib, the ideal sequencing of available KIT inhibitors will need to be clarified. In particular, for patients who are relapsed, refractory or intolerant to avapritinib, the efficacy of additional KIT inhibitors in this setting will need to be established. Targets outside of KIT that can be targeted in combination with KIT inhibitors, including antibody directed therapy targeting MCs,[65,66] intracellular signaling pathways such as JAK-STAT,[67] and BCL-2 mediating induction of apoptosis,[68] should be explored to improve upon the efficacy seen with avapritinib.

While KIT inhibition has been efficacious in controlling SM features, SM-AHN patients may continue to have complications related to the AHN. For instance, in patients with CMML (the most common AHN), reductions in bone marrow monocyte burden are minimal with midostaurin, but treatment did result in the complete normalization of eosinophilia.[69] The dynamics between neoplastic MCs and the AHN during KIT inhibition will need to be dissected in further studies. The optimal incorporation of KIT directed therapy into treatment of the AHN is also not well explored and the limitation in terms of thrombocytopenia with avapritinib introduces concurrent treatment challenges. The incorporation of KIT inhibitors associated with less myelosuppression may allow exploration of the concurrent treatment for both the SM and the AHN component.

Given the impressive activity of selective KIT inhibition in reducing and in many cases eliminating *KIT*D816V mutational burden, the concept of measurable residual disease (MRD) may now be relevant to AdvSM patients. Exploration of the predictive potential of *KIT* 816V responses for survival and incorporation of MRD into established response criteria will be important as therapeutic advances in AdvSM continue. Finally, treatment outcomes of high-risk patients, including patients with MCL, remain inadequate and further therapeutic advances are urgently needed to improve outcomes in these patients. Thanks largely to collaborations between academia and the pharmaceutical industry as well as patient advocacy groups, the increased attention directed towards this rare disease will continue to propel therapeutic advances that can improve the quantity and quality of life for patients with AdvSM.

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.

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REVIEW

Newer Modalities and Updates in the Management of Sickle Cell Disease: A Systematic Review

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Abstract: Sickle cell disease (SCD), the most common autosomal recessive genetic disorder, affects the hemoglobin (Hb) chains in human red blood cells. It is caused by mutations in the β-globin genes, leading to the production of hemoglobin S, which results in the formation of sickle-shaped red blood cells (RBCs). These abnormal cells cause hemolysis, endothelial damage, and small vessel occlusion, leading to both acute and long-term complications. According to the World Health Organization's 2008 estimates, SCD affects approximately 2.28 per 1000 individuals globally. Despite this high prevalence, therapeutic advancements have been slow. For many years, the only FDA-approved medications for managing SCD complications were hydroxyurea and deferiprone. However, recent years have seen the approval of several new therapies, including L-glutamine (2017), voxelotor and crizanlizumab (2019), as well as exagamglogene autotemcel (Casgevy) and lovotibeglogene autotemcel (Lyfgenia) (2023). These treatments have proven effective in managing both the acute and chronic effects of SCD, including hemolytic anemia, chronic pain, stroke, vaso-occlusive crises, and multiple organ damage syndromes. This review explores the mechanisms of action, practical considerations, and side effects of these emerging therapies, drawing from a comprehensive search of databases such as PubMed, Medline, and Cochrane.

Keywords: sickle cell disease, L-Glutamine, Voxelotor, Crizanlizumab, Casgevy, Lyfgenia

Introduction

Sickle cell disease (SCD) mainly affects people of African, Mediterranean, Middle Eastern, and South Asian descent, while it can strike any ethnic group.[1,2]

Because of the autosomal recessive inheritance pattern, the disease requires two copies of the faulty gene, one from each parent, to appear. Genetic tests or neonatal screening are usually used to diagnose it.

The primary intervention for anemia is blood transfusion, which increases the oxygen-carrying capacity of the blood and reduces the percentage of sickled cells. However, for vaso-occlusive crises (VOCs), the interventions include pain relief (typically with analgesics), hydration (to reduce blood viscosity), and, in some cases, blood transfusions to reduce the concentration of sickle cells and prevent further sickling.[2]

While gene editing and bone marrow transplantation (BMT) represent significant advancements in the treatment of SCD, they are not universally accessible or without significant risks. The availability of these treatments is limited by various factors, including cost, healthcare infrastructure, and patient eligibility. Moreover, while these treatments offer the potential for a cure, they come with substantial risks of morbidity and mortality, particularly in the context of BMT.[2]

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Pathophysiology of Sickle Cell Disease

(SCD) occurs due to a mutation in the hemoglobin-producing gene. Thus Hemoglobin S, an abnormal form of hemoglobin, is produced as a consequence of this mutation due to which RBCs take sickle shape when they become deoxygenated due to the polymerization of HbS molecules.[3,4] These sickled cells can induce tissue damage, occlusions in small blood vessels, and blood flow obstruction due to their stiffness and clotting tendency. Anemia results from sickled red blood cells' reduced lifespan compared to healthy red blood cells.[4]

The blockages and reduced oxygen delivery result in episodes of acute pain, tissue ischemia, and organ damage. Further, repeated sickling and unsickling cycles damage the cell membrane, contributing to chronic inflammation and endothelial dysfunction. The various consequences of SCD, such as acute chest syndrome, stroke, pain crises, and multi-organ damage, are caused by the combined effects of these processes.[4]

Past Treatment Modalities

Moderate but steady progress has been made in the development of SCD therapy techniques. Previously, care focused on symptomatic alleviation and reducing complications because there was no curative treatment available (Figure 1).[5–7] Early methods for treating anemia and vaso-occlusive crises included blood transfusions, pain relief, and hydration. Hydroxyurea became a significant medication, showing promise in lowering the frequency and intensity of the situation by increasing the fetal hemoglobin levels. However, worries about its long-term safety and some patients' poor reactivity highlighted the need for different approaches.[6]

In the past, blood transfusion has played a crucial role in sickle cell disease treatment plans by assisting in the management of the disease's numerous clinical symptoms. Transfusions, particularly those containing erythrocytes with hemoglobin A, have been shown to increase hemoglobin levels, lower the risk of anemia, and reduce the incidence of stroke. Transfusion methods such as red blood cell exchange, simple transfusion, and chronic transfusion have been employed to tailor therapy regimens to individual patient requirements.[7]

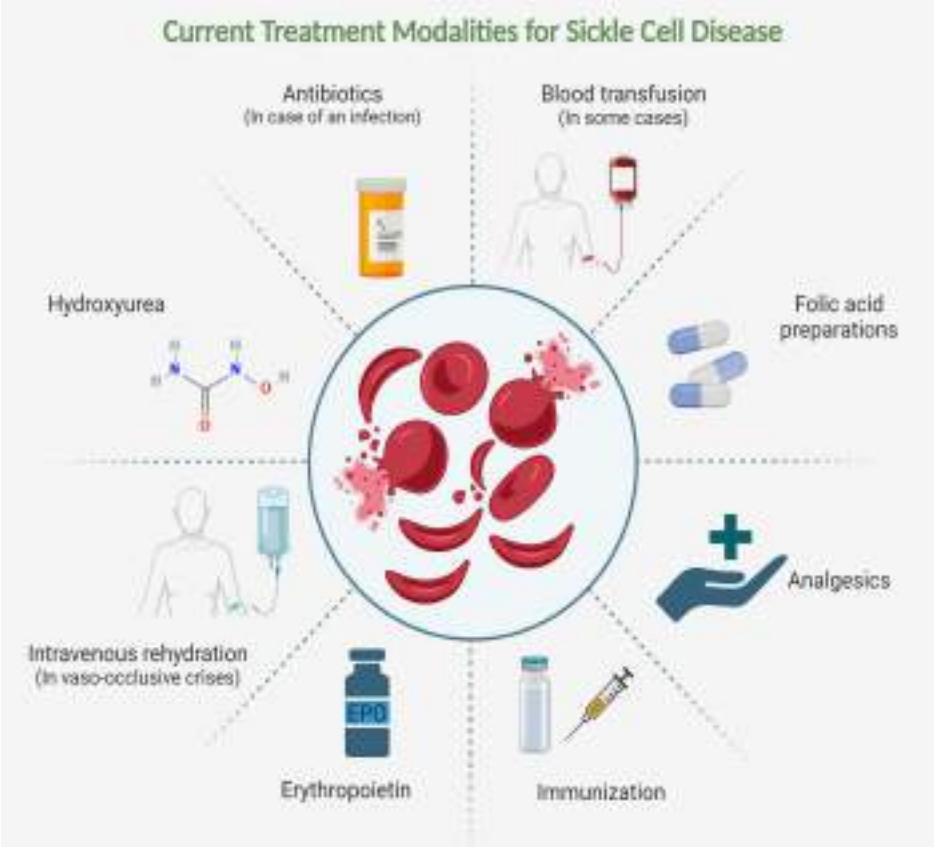

Figure 1 Current and past treatment modalities for patients diagnosed with Sickle Cell Disease.

Even though previous treatment modalities have helped SCD patients live better lives and achieve better outcomes, more work is needed to address the problems that still exist, such as disparities in treatment availability, access to care, and the search for curative interventions to lessen the disease's significant burden.

Limitations of Previous Treatment and Need for New Treatment Modalities

Regarding the development of new frontline treatments for SCD, it has historically grappled with significant limitations. Thus, there has been a pressing need for novel treatment modalities. Previous treatments like fluid replacement, pain relief, and blood transfusions attempted to relieve symptoms, but they frequently failed to address the disease's underlying pathophysiology. Hydroxyurea has proven to be an effective treatment for SCD due to its ability to increase fetal hemoglobin levels, thereby reducing vaso-occlusive crises.[8] However, its inconsistent response rates and worries about long-term safety and adherence brought to light ongoing gaps in available treatments. For some people, hydroxyurea therapy does not produce sufficient results. For these patients, the disease may continue to progress despite treatment, requiring new or different therapeutic modalities. Moreover, hydroxyurea alone might not be sufficient to prevent or treat all SCD complications, including cerebral vasculopathy.[8] L-glutamine was introduced in the following years as an oral preparation, which acts via a novel mechanism of action by lowering oxidative stress and promoting cellular detoxification. A hemoglobin polymerization inhibitor, namely Voxelotor, recently got approval, which was considered a major step because it gave people with hemoglobinopathies a targeted treatment option. The development of new molecules such as Voxelotor and Crizanlizumab, as well as advancements in gene therapy, offer promise as good treatment options. Voxelotor's development as an inhibitor of hemoglobin polymerization represents a breakthrough that provides targeted treatment for people with hemoglobinopathies.[9] Ongoing assessment is necessary, though it has shown promising results for its long-term safety profile and effectiveness in larger populations. A comprehensive strategy addressing both immediate complications and long-term effects, such as organ damage and reduced quality of life, is necessary due to the complex nature of sickle cell disease.[8,10] To improve outcomes and quality of life for affected individuals, it has been urgently necessary to develop novel treatment modalities that not only address the intricate interactions between clinical manifestations but also target the underlying cause of SCD.

Methodology

Literature Search Method

For the conduction of a comprehensive review, we adhered to the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines" (Figure 2).

A thorough search was carried out on electronic databases such as Pubmed, Medline and Cochrane and search strategy was developed using a combination of keywords and Medical Subject Headings (MeSH) terms such as "sickle cell disease", "sickle cell anemia", "sickle cell crisis", "L-Glutamine", "Endari", "Crizanlizumab", "P-Selectin inhibitor", "Voxelotor", "Oxbryta", "hemoglobin S polymerization inhibitor", "Exagamglogene autotemcel", "Casgevy", "Lovotibeglogene autotemcel", "Lyfgenia", "CRISPR/CAS9", "lentiviral gene therapy" and related terms.

Inclusion Criteria

Randomized controlled trials and clinical trials that were relevant to treatment outcomes and adverse effects of the above drugs for the management of SCD were included in the review. Only the articles in the English language were considered.

Exclusion Criteria

We excluded review articles, letter-to-editor, abstracts, and studies that were not directly related to recently approved drugs.

Results

We included 23 articles in total which were relevant to new drugs and the patient treatment outcome. In our paper, we discussed L-Glutamine, a potent antioxidant agent and Voxelotor, an anti-sickling drug that had shown promising results in pain reduction and improving haematological profile in all age groups of people including paediatrics, Crizanlizumab,

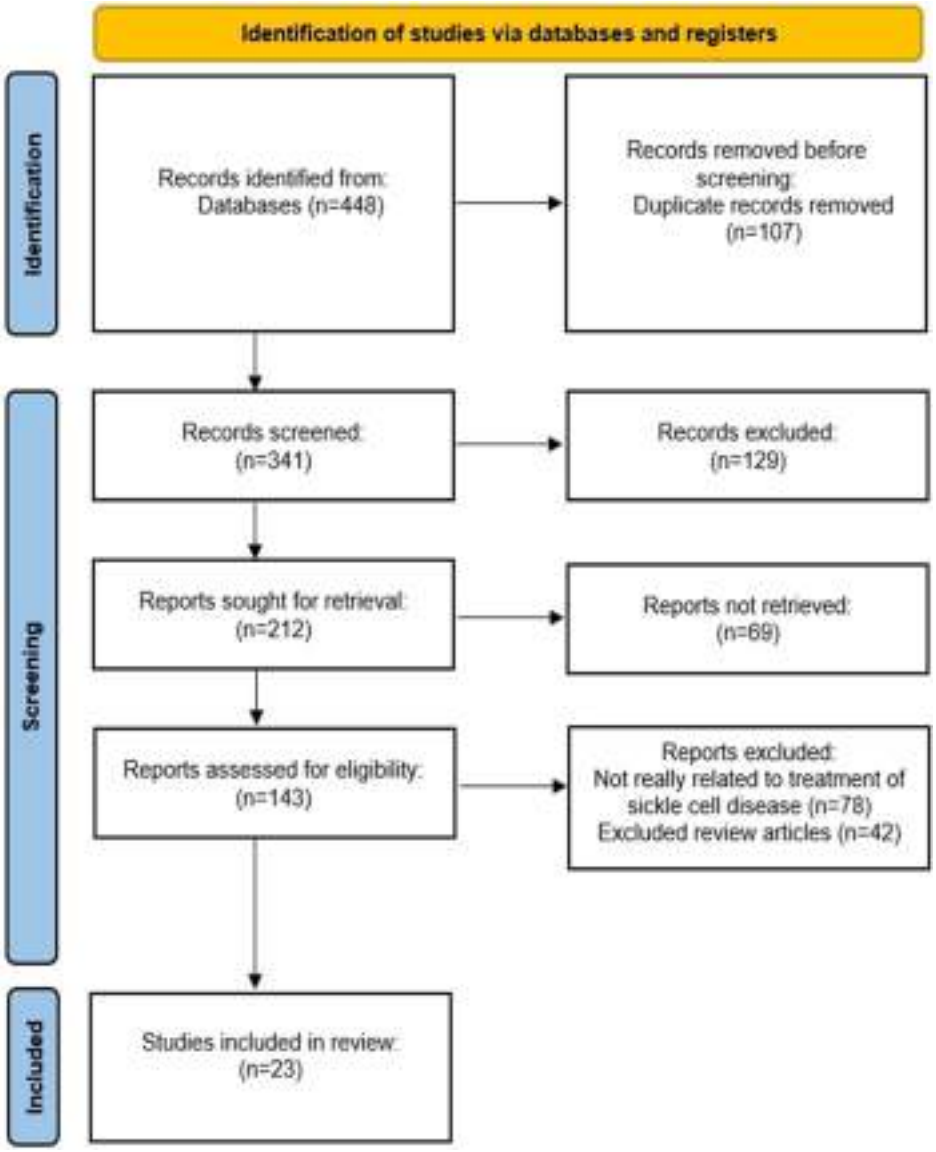

Figure 2 Prisma Flow Diagram.

a P-Selectin inhibitor which alleviates vaso-occlusive crisis and evolving gene therapies like Exagamglogene autotemcel-(Casgevy) and Lovotibeglogene autotemcel (Lyfgenia). Casgevy and Lyfgenia are CRISPR/Cas 9-based groundbreaking therapies to treat SCD from its origin.

The summary of included text is given below in table (Table 1).

Table 1 Shows Summary of the Included Studies

Author's last name-year	Type of study	Objective	Conclusion
Gotesman et al 2022[11]	Retrospective cohort study	To find out if a healthy diet and L-glutamine (Gln) could reduce the severity of sickle cell anemia	Younger, healthier, and better-hydrated patients typically have more favorable clinical outcomes. The severity of the sickness was highest among teenagers who did not follow proper diet and hygiene habits. L-glutamine along with pre-albumin monitoring should be considered for additional evaluation in pediatric SCD.

(*Continued*)

Table 1 (Continued).

Author's last name-year	Type of study	Objective	Conclusion
Elenga et al 2023[12]	Prospective cohort study	To evaluate the overall effect of L-glutamine therapy on kidney function in patients with sickle cell disease.	According to the study, L-glutamine therapy improved therapeutic outcomes and decreased hemolysis. Throughout 48 to 120 weeks of treatment, it was found that L-glutamine improved kidney function in patients with sickle cell disease.
Vichinsky et al 2019[13]	Phase 3 Randomized control trial	To evaluate voxelotor in people suffering from SCD	Voxelotor significantly increased hemoglobin levels in SCD patients while lowering the risk of hemolysis and exacerbating their anemia. Following voxelotor treatment, hematologic improvements were noted, and concurrent HU treatment may have an added benefit.
Muschick et al 2022[14]	Retrospective cohort study	To examine general quality-of-life outcomes and the hematologic response in patients receiving voxelotor treatment.	Following voxelotor treatment, hematologic improvements were noted, and concurrent HU treatment may have an added benefit.
Estepp et al 2022[15]	Phase 2a clinical trial	Evaluate the voxelotor's effectiveness and safety in pediatric SCD patients (4–11 years old).	Voxelotor significantly improved hemolytic markers and Hb levels, demonstrating efficacy consistent with adult and juvenile SCD patients. The safety profile was good, with few unfavorable situations that required stopping. Dispersible pill dosing based on weight was well tolerated.
Shah et al 2022[16]	Retrospective analysis of the database	Evaluate the efficacy of Sickle cell disease Voxelotor in treating sickle cell disease in the United States.	Based on the evidence of improved hemoglobin levels, lower transfusion rates, shorter hospital stays and VOC-related hospitalizations, among other outcomes, the study proposes that V, oxelotor may reduce transfusion and vaso-occlusive crisis (VOC) rates in clinical practice.
Hutchaleelaha et al 2019[17]	Phase 1/2 Randomised clinical trial	To assess Voxelotor's (GBT440) pharmacokinetics and pharmacodynamics in sickle cell disease patients and healthy adults.	With a once-daily dosage, Voxelotor (GBT440) exhibited prolonged pharmacodynamic effects due to its high binding specificity for hemoglobin and linear pharmacokinetics across the investigated dose range. The study demonstrated that Voxelotor was well tolerated in both sickle cell disease patients and healthy volunteers, and it offered evidence of a mechanism for boosting Hb-oxygen affinity.
Howard et al 2019[18]	Phase 1/2 Randomised clinical trial	To evaluate Sickle cell disease Voxelotor's pharmacokinetic and pharmacodynamic characteristics in sickle cell disease patients.	In sickle cell disease patients, Voxelotor had encouraging pharmacokinetic and pharmacodynamic profiles, indicating the drug's potential as a therapy alternative.
Phan et al 2023[19]	Clinical trial	Examine how voxelotor affects cardiopulmonary testing in young people with	In 9 out of 10 young SCA patients already on HU with reasonably high Hgb F, Voxelotor therapy did not increase peak VO2. As anticipated, Voxelotor increased hemoglobin levels, but it also changed the hemoglobin oxygen dissociation curve. The lack of improvement in exercise was likely caused by limitations in oxygen delivery.

(*Continued*)

Table 1 (Continued).

Author's last name-year	Type of study	Objective	Conclusion
Alshurafa et al 2022[20]	Case report	Voxelotor safety and effectiveness in a patient with stage IV chronic renal disease and sickle cell disease	In summary, based on the available data, this case report suggests that voxelotor is safe and tolerated in patients with sickle cell disease (SCD) and severe renal impairment. However, more research is needed to corroborate this result.
Strader et al 2019[21]	Case report	To assess the selectivity and stoichiometry of GBT440-HbS adducts in a hemolysate from a patient with sickle cell disease.	The hemolysate of SS patients contains GBT440-HbS adducts that are unique to the α subunit and occur in a 1:1 stoichiometry, which helps with dose optimization and therapy monitoring.
Kanter et al 2023[22]	Phase 2 open-label clinical trial	Examine crizanlizumab's safety, PK/PD, and effectiveness in patients with sickle cell disease (SCD) to gauge leukocyte adherence to P-selectin and the drug's inhibitory effect.	Crizanlizumab reduces VOC frequency effectively and has a good safety profile at 5.0 mg/kg. PK/PD profiles that are in line with earlier research.
Man et al 2020[23]	Short communication review article	To assess actual Crizanlizumab data in SCD	The assay for standardized microfluidic biochip whole blood adhesion showed that leukocytes adhered heterogeneously to immobilized P-selectin and that this adherence was inhibited in a dose-dependent manner after pre-exposure to crizanlizumab. Crucially, Crizanlizumab therapy induced rolling leukocyte dissociation but did not firmly adhere to leukocytes after attachment to P-selectin. It is proposed that the microfluidic Biochip technology is a promising in vitro assay for SCD patient screening, treatment response monitoring, and guiding the development of new and existing anti-adhesive medicines.
Cheplowitz et al 2023[24]	Retrospective analysis of the database	To look at DMT use in SCD patients from 2014 to 2021 and assess patterns and traits	Crizanlizumab may reduce the number of acute care visits, especially for heavy users; nonetheless, the high rate of discontinuation suggests that more research is required.
Newman et al 2023[25]	Cross-sectional study	Evidence to date about the profile of crizanlizumab and its potential to prevent pain crises in sickle cell disease	The study discovered a gradual rise in the usage of DMT, especially with more recent treatments like crizanlizumab and voxelotor. Still, there is a significant unmet need in the SCD community as seen by the low total DMT use.
Riley et al 2019[26]	Review article	To assess LentiGlobin's effectiveness and safety in sickle cell disease patients	According to phase 2 studies, crizanlizumab has a favorable safety profile and may help people with sickle cell disease have fewer vaso-occlusive crises.

(*Continued*)

Table 1 (Continued).

Author's last name-year	Type of study	Objective	Conclusion
Kanter et al 2022[27]	Phase 1–2 clinical trial	To assess LentiGlobin's effectiveness and safety in sickle cell disease patients	After receiving LentiGlobin for a single treatment, antisickling hemoglobin was produced continuously, which resolved the severe vaso-occlusive episodes and produced a safety profile in line with the known hazards associated with autologous stem-cell transplantation. There were no documented cases of cancer or stroke, and the demographics were typical of the larger sickle cell disease population in the US. A small sample size, a brief follow-up period, and the absence of a control group are among the limitations. Sustained monitoring is required to evaluate long-term safety and efficacy.
Kanter, Thompson et al 2022[28]	Phase 1/2 open-label Clinical Trial	To assess the development of lovo-cel gene therapy as a treatment and its results for sickle cell disease	The study shows how the course of treatment has changed and how the HGB-206 trial groups have fared well.
Drakopoulou et al 2022[29]	Cohort study	The goal of the study was to ascertain whether sickle cell disease (SCD) CD34+ cells could be cultured in vitro to produce higher amounts of fetal hemoglobin (HbF) and lower levels of sickle hemoglobin (HbS) using the GGHI-mB-3D lentiviral vector.	In SCD, the use of CD34+ cells cultured in the GGHI-mB-3D lentiviral vector has been shown to increase HBF levels and decrease HBS levels, suggesting its use as a therapeutic intervention for SCD.
Morgan et al 2020[30]	Lab-based experiment	To assess a new lentiviral vector design for sickle cell disease (SCD) gene therapy.	The use of CoreGA-AS3-FB in a mouse model of SCD showed better infectivity and therapeutic efficacy as a therapeutic intervention for SCD.
Weber et al 2020[31]	Lab-based experiment	To repair the sickle cell disease phenotype and restore fetal hemoglobin synthesis by editing a binding site of γ-globin repressor.	The study demonstrated that the phenotype of SCD can be corrected and HBF synthesis can be restored by editing the γ-globin repressor binding site, suggesting a role of gene editing as a treatment option for SCD patients.
Koniali et al 2023[32]	Lab-based experiment	Testing the safety and effectiveness of gene therapy for sickle cell disease utilizing a GLOBE1 lentiviral vector-transduced autologous CD34+ enriched cell fraction.	The study demonstrated the positive effectiveness and safety of gene therapy (GLOBE1 lentiviral vector) as a treatment of SCD.
Galactéros et al 2023[33]	Meta-analysis	To determine the effect of voxelotor on the burden of sickle cell disease using a modelling technique in France and considering the device's expected acceptance and dispersal over the next five years.	Voxelotor may benefit public health by enhancing haemoglobin levels in SCD patients when used as a treatment choice and lessening the toll of SCD on individuals and the medical system.

Discussion

Novel approaches to treating sickle cell disease can target different pathophysiological pathways. The dysregulation of the von Willebrand factor (VWF) - ADAMTS13 axis is a key component of the pathophysiology of sickle cell disease and plays a significant role in its pathogenesis.[34] Examining the effects of oxidative stress and chronic hemolysis, which aggravate organ damage and endothelial dysfunction, is another area of research. Through the reduction of hemolysis,

oxidative stress, and inflammation, these treatments seek to slow down the course of the disease. Furthermore, the goal of therapies targeting sickle cell adhesion to the vascular endothelium and endothelial dysfunction is to avoid tissue ischemia and vaso-occlusion.[35] Another important pathophysiological event in sickle cell disease is the polymerization of hemoglobin under deoxygenation, which causes red blood cell sickling and other complications. As a result, it has become a key objective to stop sickle hemoglobin (HbS) from polymerizing, which will stop sickle-shaped red blood cells from forming and lower the risk of vaso-occlusive crises. Furthermore, methods to enhance the synthesis of fetal hemoglobin (HbF) have attracted interest because HbF prevents HbS polymerization and lessens the symptoms of SCD.[36] Since inflammation is essential to both chronic organ damage and vaso-occlusive crises, it also presents a target for intervention. It is possible that treating inflammation and its consequences will help SCD patients' live better lives.[36]

L-glutamine aims to lower the burdens associated with this disease by acting through various pathways. L-glutamine operates by replenishing cellular antioxidants. Thus by enhancing the antioxidant defenses within cells, it helps to overcome damaging effects of reactive oxygen species. L-glutamine also enhances the production of nitric oxide, a pivotal molecule involved in vasodilation.[11] Gotesman et al[11] in a retrospective cohort study, found that younger, healthier, and better-hydrated patients had more favorable clinical outcomes. The study suggests that L-glutamine, along with pre-albumin monitoring, should be considered for additional evaluation in pediatric SCD patients. In addition, Elenga et al[12] prospective cohort study concluded that L-glutamine therapy improved therapeutic outcomes and decreased hemolysis, particularly enhancing kidney function over 48 to 120 weeks of treatment in SCD patients. By facilitating vasodilation, L-glutamine contributes to improving vascular tone and ameliorating complications associated with impaired blood circulation. It also exhibits modulatory effects on inflammatory pathways by dampening inflammatory responses, it helps mitigate the systemic inflammation characteristic of the disease.[11,12]

Treatment with L-glutamine has shown positive outcomes in the control of SCD. According to studies, giving L-glutamine therapy results in a lesser number of hospital admissions, fewer red blood cell transfusions, a lesser number of pain crises, and longer intervals between first and second crises. It has also been found that L-glutamine significantly reduces the need for vaso-occlusive crises (VOCs), acute chest syndrome (ACS), hospital stays, and blood transfusions. Additionally, it has been shown that L-glutamine treatment improves clinical outcomes through a decrease in hemolysis markers, an increase in hemoglobin levels, and a reduction in hospital admissions, days of stay, and VOCs.[37,38]

In an article recently published by Narcisse, Elenga, Gylna, Loko et. al[37] on real-life data for L-glutamine therapy, Glutamine has shown remarkable efficacy in alleviating various clinical manifestations of SCD, as proved by several key findings from the study evaluating its impact. Treatment with L-glutamine resulted in an observable reduction in the number of pain crises, hospitalizations, days of hospitalization, and blood transfusions among patients with SCD at 24, 48, and 72 weeks following initiation of therapy.

The mechanism of action of Voxelotor involves blocking the polymerisation of haemoglobin.[19] This is achieved by binding to sickle haemoglobin (HbS) and stabilising it in the oxygenated state leading to an overall decrease in the formation of HbS polymer, thereby reducing sickling of red blood cells (Figure 3). Furthermore, the reduced viscosity and lesser deformability of the red blood cells improves blood flow and it results in fewer incidences of vaso-occlusive crises.[17] The inhibition of HbS polymerization may also lessen hemolysis, oxidative stress, and endothelial dysfunction. These factors combined together further enhance the overall outcomes of Voxelotor use in SCD.[18,20]

Vichinsky et al[13] in a phase 3 randomized control trial, voxelotor was shown to significantly increase hemoglobin levels in SCD patients while lowering the risk of hemolysis and exacerbating anemia. The study highlighted hematologic improvements with possible additive benefits when used with hydroxyurea (HU). In addition, Shah et al[16] in their retrospective analysis, highlighted voxelotor's potential to reduce transfusion rates, hospital stays, and VOC-related hospitalizations in clinical practice. Also, Hutchaleelaha et al[17] A phase 1/2 trial showed voxelotor's prolonged pharmacodynamic effects due to its high binding specificity for hemoglobin, with a good tolerance profile.

OxbrytaTM, commonly known as voxelotor, is a game-changer in the treatment of sickle cell disease. Its distinct process entails attaching to hemoglobin, maintaining its oxygenated form, and preventing HbS polymerization, which holds great promise for individuals suffering from this painful sickness.[40,41] With once-daily oral administration, it unleashes a barrage of benefits, from reducing red-cell sickling and improving blood viscosity to enhancing red-cell deformability.[18,42] Moreover, its knack for extending red-cell half-life and curbing anemia in vivo underscores its

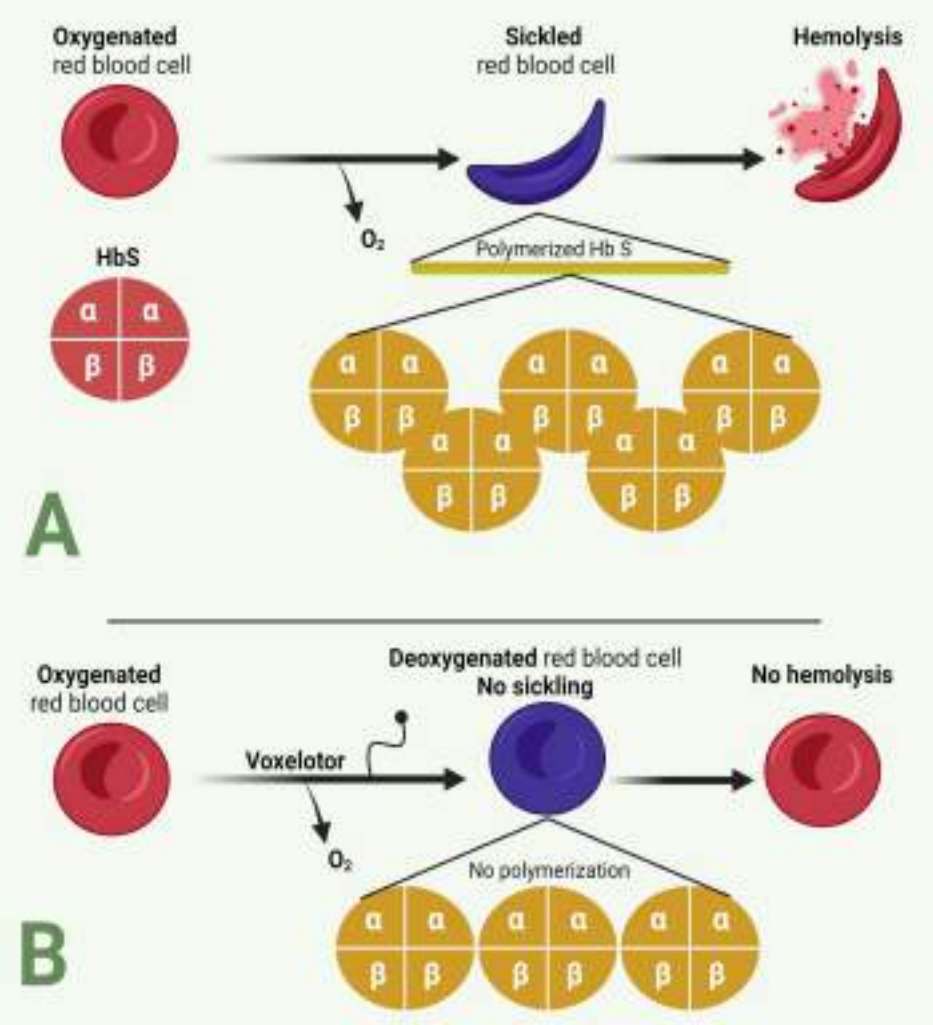

Figure 3 The mechanism underlying sickle cell disease and the therapeutic action of Voxelotor in preventing the sickling of red blood cells (RBCs).
Notes: (**A**) The process begins with an oxygenated red blood cell, where hemoglobin is in its normal soluble form. As the cell becomes deoxygenated, the abnormal hemoglobin S (HbS) molecules polymerize, forming long, rigid chains that distort the cell into a sickle shape. This sickling of RBCs makes them less flexible and more prone to hemolysis. (**B**) Voxelotor exhibits preferential binding to hemoglobin, enhancing its affinity for oxygen, hence maintaining it in the oxygenated state and inhibiting sickling. Data from Engel ER, Howard AL, Ankus EJ, Rico JF. Advances in Sickle Cell Disease Management. Adv Pediatr. 2020 Aug;67:57-71. doi: 10.1016/j.yapd.2020.03.001. Epub 2020 May 14. PMID: 32591064.[39]

potential to revolutionize sickle cell disease management.[13,43] Although concerns initially loomed over potential oxygen delivery compromises, preclinical findings put those worries to rest, emphasizing voxelotor's knack for maintaining tissue oxygenation without compromising organ function.[41,44,45] Furthermore, voxelotor emerges as a ray of hope for people living with sickle cell disease, providing not just alleviation but also a fresh sense of hope, because of clinical studies such as the phase 3 HOPE trial that are highlighting it.[42] Subjects using oxelotor during trials reported few major or severe side effects after 6 months of treatment.[46] More than 10% of cases were of diarrhea, nausea, vomiting, stomach discomfort, fever, rash, headache and were the most frequently reported adverse effects while less than 1% of people experienced anaphylaxis, making it an uncommon occurrence.[47–49] No adverse effects specific to cardiovascular or respiratory function were identified.[50] Trials conducted thus far have been small in size and have not included individuals with other significant comorbid health conditions. Additional adverse effects may be reported as more individuals gain access to voxelotor and postmarketing data are available. Voxelotor may affect laboratory measurements, interfering with high-performance liquid chromatography readings of hemoglobin variants (HbA, HbS, HbF).[43] Individuals and providers can temporarily discontinue voxelotor for accurate subtype readings, as its effect is not permanent. The medication is contraindicated only in cases of documented hypersensitivity.[17] Limited data exist regarding voxelotor's use in pregnant women and breastfeeding mothers, with caution advised due to potential adverse effects on infant hematopoiesis.[13] Adolescents aged 12 and older can take voxelotor, but data on its use in individuals aged 65 and older are lacking.[51]

Nurses should counsel women with SCD on contraception options, considering the risks of SCD during pregnancy.[52,53] In a single-center study of Kathryn Muschick et al[14] Voxelotor treatment showed favorable hematologic responses in patients with SCD like increased hemoglobin (Hb) levels, decreased reticulocyte percentage, and reduced total bilirubin. The study included 77 patients, mostly female (62%) with homozygous HbSS genotype (86%) and concomitant hydroxyurea (82%). Voxelotor demonstrated potential additive benefits when used with hydroxyurea, enhancing hematologic improvements. Adverse events were rare, mild, and resolved with dose modification. Quality-of-life outcomes improved, assessed via patient and clinician global impression questionnaires, with higher scores in patients using hydroxyurea. This retrospective review, although limited by its observational nature and single-center design, provides valuable real-world insights into the efficacy of voxelotor in treating SCD.[54]

Crizanlizumab works as a P-selectin inhibitor, targeting important mechanisms of vaso-occlusive events. P-selectin is an adhesion molecule found on endothelial cells and platelets, it is responsible for the adhesion of red blood cells to the vascular endothelium.[22,23] P-selectin then facilitates the RBCs' subsequent entrapment and occlusion within the microvasculature. Crizanlizumab binds to P-selectin and covers its active site thereby preventing the interaction between RBCs, platelets and endothelial cells. This effectively lowers the frequency and severity of vaso-occlusive crises.[23,25] Crizanlizumab's therapeutic benefits also involve an additional mechanism that works by reducing inflammation and endothelial activation. Kanter et al[22] a phase 2 open-label clinical trial found that crizanlizumab effectively reduced VOC frequency with a good safety profile. A cross-sectional study by Newman et al[25] showed a gradual rise in crizanlizumab usage, highlighting its potential in preventing pain crises in SCD, though unmet needs remain in the SCD community.

VOCs are a major cause of morbidity and mortality in individuals with SCD and often lead to hospitalizations and decreased quality of life. The landmark SUSTAIN trial demonstrated the efficacy of crizanlizumab in reducing the frequency of VOCs among patients with SCD. Crizanlizumab causes sickle cells, endothelial cells as well as leukocytes to not adhere to one another by selectively inhibiting P selectin. In contrast to volatile organic molecules, this lessens the inflammatory reaction and microvascular obstruction.[55] The pathophysiology of SCD is consistent with this method of action as the tissue damage and organ dysfunction are caused by ischemia reperfusion injury and vaso-occlusion.

Moreover crizanlizumab ability to prevent VOCs extends beyond symptom management to address the underlying pathophysiology of SCD. P selectin mediated platelet activation and endothelial cell adhesion play critical roles in the initiation and propagation of VOCs. Crizanlizumab disrupts these processes and thereby reduce the incidence and severity of VOCs. This targeted approach offers a novel therapeutic strategy for individuals with SCD and potentially minimizes the need for opioid analgesics and hospitalizations associated with VOCs.[56] The safety and tolerability profile of Crizanlizumab observed in clinical trials further support its potential as a disease modifying therapy for SCD.

In addition to its effects on VOCs, Crizanlizumab may also have broader implications for other SCD related complications. ACS, a severe pulmonary complication of SCD, shares common pathogenic mechanisms with VOCs including inflammation and endothelial activation and and microvascular occlusion. Preclinical studies have shown that P selectin blockade can attenuate lung injury and improve outcomes in mouse models of ACS, suggesting a potential role for Crizanlizumab in preventing or mitigating this complication in individuals with SCD.[57] Furthermore, the antithrombotic properties of Crizanlizumab may reduce the risk of thrombotic events in patients with SCD who are predisposed to both venous and arterial thrombosis.[58] By targeting P selectin, Crizanlizumab offers a multifaceted approach to managing SCD and addressing both acute and chronic complications associated with the disease.

Autologous HSCT refers to when stem cells are obtained from the patient's marrow or blood. These can then be altered in laboratory settings so that they lack any genetic abnormalities found therein before being reintroduced back into the donor's body system again. Although this method eliminates compatibility issues with donors, it does not necessarily work for all diseases. Morgan et al[30] in their lab-based experiment, showed that the CoreGA-AS3-FB lentiviral vector improved therapeutic efficacy in a mouse model of SCD, offering a promising gene therapy approach. Gene editing is a novel technique that allows scientists to alter an organism's nucleotide sequence directly at a highly specific point. This offers hope in finding treatment for sickle cell disease, which is also a genetic disorder. Weber et al[31] which is another lab-based experiment demonstrated the correction of the SCD phenotype and restoration of HbF synthesis through gene editing, highlighting the potential of gene therapy for SCD patients.

Challenges and Further Perspectives

Even with the introduction of disease-modifying medications such as L-glutamine oral powder, Voxelotor, and Crizanlizumab and the use of stem cell transplantation in certain situations, issues like restricted availability, related hazards, and insufficient effectiveness highlighted the necessity for more thorough and easily accessible therapeutic methods.

L-glutamine treatment has been associated with side effects such as abdominal pain, nausea, vomiting and serious side effects such as spleen enlargement, indigestion, and hot flashes.[59]

Patients receiving voxelotor have common side effects due to medication, such as pyrexia, diarrhea, vomiting, headaches, and back discomfort.[60] Also, adverse effects like the pulmonary crisis were reported by Vichinsky et al which was stated "may be unrelated" to Voxelotor the author.[13]

The major side effects that were observed after the therapeutic use of crizanlizumab were pyrexia, influenza, and pneumonia along with some occasionally reported side effects like headache, nausea, back pain, chest discomfort, and arthralgia.

Gene therapy and stem cell transplantation are considered promising approaches for treating SCD. However, its implementation on a large scale is still limited due to several reasons. First of all, finding compatible donors for allogeneic hematopoietic stem cell transplants can be difficult for many patients, making the very first step a significant challenge, Furthermore, the high expense of gene therapy and stem cell transplantation overburdens patient pocket expenditure. The limitation to access such therapies is also a significant barrier to people, especially for those living in resource-constrained environments.

Even when it comes to treating pediatric populations with SCD, stem cell transplantation has become the standard of excellence, but its use is limited by the risks involved and the availability of suitable donors.

Moreover, considering gene therapy and gene editing techniques are still in their early stages of development, concerns about safety and efficacy are yet to be tested. Further research and rigorous clinical trials are necessary to establish the safety and effectiveness of these innovative approaches before they are widely implemented as standard treatments for SCD.

Limitations

Although this systemic review was carried out with utmost precision, there might be a deficiency in data due to limited trials and studies available. Furthermore, the data is available for a limited population which might hinder the quality of information available. The papers included in our study were in English language only, so the possibility is there that we might have missed important papers.

Conclusion

Over the past ten years, advancements in sickle cell disease have given doctors more alternatives and opened the gateway for more studies. The pathophysiology of disease advancement and the therapies that regulate its progression are now more understood. There has been rapid advancement in the discovery of variable solutions available that have the potential to treat the underlying cause of the symptoms and alleviate them.

The FDA approval of Exagamglogene autotemcel and lovotibeglogene autotemcel, which treat diseases at their base, would undoubtedly change the game. Additionally, a sizable number of individuals from nations with both abundant and scarce resources who are unable to pay for stem-cell therapy would benefit from L-glutamine, Voxelotor, and Crizanlizumab.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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A Dosimetric Comparison Study for Blood Irradiation Employing Different Medium and Algorithms in Clinical Linear Accelerator

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A Dosimetric Comparison Study for Blood Irradiation Employing Different Medium and Algorithms in Clinical Linear Accelerator

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Objective: To identify a suitable approach for blood irradiation other than the commonly used water medium and to study the impact of different algorithm dose computations.

Methods: Water is the commonly used medium for blood irradiation. In this study computed tomography scans were taken with locally made blood irradiation phantoms other than water, by using air, rice powder and thermocole using parallel beam for 25 Gy. Plans were recalculated for different algorithms such as collapsed cone (CC), Monte Carlo (MC) and pencil beam (PB). The dose–volume parameters and measured doses were collected and analyzed for each medium and algorithm.

Findings: The monitor unit (MU) for rice powder and water are close (2461±57 and 2469±61, respectively), with a maximum dose of 28.0±1.8 and 28.0±1.9 Gy. The PB algorithm resulted in lower monitor unit values regardless of the medium used, generating values of 2418, 2406, 2382, and 2362 for water, rice powder, air, and Thermocol, respectively. A significant increase in dose was observed irrespective of the medium used when the MC algorithm was employed, with a maximum of 30.26 Gy in rice powder; a smaller dose was used when the CC algorithm was employed, with 26.3 Gy in water medium. The average maximum doses of all groups were equal using the one-way Anova statistical test. Regarding the impact of field size, rice powder appears to have consistent doses across various field sizes, with slight increases as field size grows, which is similar to water.

Novelty/Applications: While water is the conventional medium, this study highlights the potential benefits of rice powder, such as eliminating the risks associated with bubble formation and water spillage, which can lead to equipment malfunction and safety hazards. Although previous studies have explored rice powder as a bolus and tissue-equivalent material, this study uniquely applies this knowledge to blood irradiation, an area where rice powder has not been thoroughly investigated.

Keywords: computed tomography, collapsed cone, Monte Carlo, pencil beam, monitor units, MU, treatment planning station.

Introduction

TA-GVHD is a rare complication of transfusion that may occur when transfused lymphocytes are viable and one of the following is present in the recipient. The recipient is either immunosuppressed or there is a partial HLA matching between the transfused product and the recipient. Immune attack is mediated by the transfusion donor's viable T cells, either through direct destruction of host cells or via inflammatory cytokines that activate other immune cells, including natural killer (NK) cells, macrophages, and other lymphocytes. In such a scenario, blood irradiation has the potential to eliminate the ability of lymphocytes to mount an immune response by preventing its ability to replicate, serving as the sole genuinely efficient method for such disease prevention.[1] The primary approach to blood irradiation involves exposing the blood to gamma photons using specialized irradiator units containing either one of the radioactive isotopes, that is, Cobalt 60 or cesium 137.[2] Due to the unavailability of such devices in many departments, it is quite unusual to use the clinical linear accelerator machine for such procedures. Numerous research studies have indicated that the

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irradiation of blood using clinical linear accelerators is feasible, providing cost savings and allowing for the efficient utilization of existing facilities.[3,4] In our radiotherapy department we were using 6 MV photon energy Elekta Versa HD (Stockholm UK) for such an irradiation procedure. Blood irradiation is a standard practice in our department for bone marrow transplants. Our irradiation setup includes a specially designed perspex box with a lid, filled with water to deliver a 25 Gy dose uniformly, which will completely suppress the lymphocyte response.[5,6] Before the blood irradiation setup was initiated, the total monitor units were computed for the phantom using a water medium to deliver the prescribed dose at the isocenter for both anterior–posterior and posterior–anterior beams in our Monaco planning station (IMPAC Medical System, Inc., Maryland Heights, USA) using a collapsed cone (CC) algorithm. Additionally, dosimetric verification was conducted using a Semiflex chamber and Gafchromic irradiator indicator film to ensure accurate dose calculation.

Upon thorough examination of this method involving water, it becomes evident that there are several significant concerns that must be carefully addressed. The primary concern associated with the use of water in this method concerns the formation of bubbles, which could significantly impact the uniformity of dosage distribution. Another critical issue arises from the possibility of water spilling onto the machine's couch, as well as the motor and linac head, if not managed with the utmost care. Water spillage poses not only a risk to the integrity of the equipment but also introduces potential safety hazards for personnel operating the machine and patients undergoing treatment. If unnoticed, this ingress of water into sensitive components of the machine, such as the motor and linac head, can result in corrosion, electrical malfunction, and other operational inefficiencies, thereby necessitating costly repairs and down time. Given these concerns, particularly in the context of blood irradiation procedures, it becomes evident that addressing these challenges would require substantial investment. This includes the development and implementation of stringent protocols to prevent bubble formation and water spillage, alongside comprehensive training for personnel to ensure that they can manage these risks effectively. However, the complexity and cost associated with mitigating these issues make it clear that an alternative medium is highly desirable.

Rice powder can be used as a promising alternative for such procedures. Unlike water, it does not carry the risk of bubble formation or spillage. As a dry medium, it naturally eliminates the possibility of liquid-related hazards such as corrosion or electrical failure. Additionally, rice powder can provide a more uniform medium for dosage distribution, potentially improving the overall accuracy and safety of irradiation procedures. The shift to using rice powder would not only address the significant concerns associated with water but also streamline the process, reducing the need for extensive safety protocols and training while enhancing the reliability of the treatment. Numerous studies have focused on the dosimetric aspects of rice powder, examining its use as a bolus material and its viability as a tissue-equivalent material.[7–10] The results of this study consistently demonstrate the suitability of rice powder for various radiation applications, positioning it as a versatile and practical alternative to traditional water-based systems. Therefore, this study primarily investigates the viability of using rice powder as an alternative substrate, in place of water, for a faster and simplified workflow in blood irradiation.

Ensuring precise calculations is another crucial aspect of radiation therapy. The planning station employs various mathematical formulations and algorithms to achieve swift and accurate dose calculations. An optimal dose calculation algorithm can accurately represent the real dose distribution, thus minimizing uncertainty in plan evaluations. Different algorithms produce varying results when calculating in an inhomogeneous medium, with a more noticeable impact on computation compared to the changes observed in a homogeneous medium. Currently, various vendors are offering distinct algorithms for dose calculation. These algorithms operate using pre-set input radiation beam data obtained during machine commissioning. In our department, we have Monaco™ V 5.11 TPS for external beam radiotherapy treatment planning, which have PB algorithm, CC and MC for dose calculation. The CC algorithm employs various simplifications in the physics of radiation transport, allowing for computation times suitable for clinical application. In contrast, the pencil beam (PB) algorithm is highly efficient but has limitations in heterogeneous media due to utilizing a one-dimensional density correction, which fails to accurately capture the distribution of secondary electrons in materials with differing densities. The MC algorithm, in contrast, is presently considered to be one of the most sophisticated for dose computation.[11,12]

Existing research has primarily focused on computational algorithms and their performance, with limited investigations into the effects of different medium on blood irradiation. This study aims to fill this gap by comparing three distinct dose-calculation algorithms, namely, PB, CC, and MC, provided by a commercial treatment planning system, to assess their impact on various substrates for blood irradiation. Additionally, the study analyzes the influence of field size in conjunction with the medium. The study was conducted at our radiation oncology department using an Elekta HD versa linear accelerator and Monaco Planning station.

Materials and Methods

The study was conducted using a custom-built phantom at our hospital, following approval from the Institutional Research Committee of Kasturba Medical College and Kasturba Hospital. The Perspex phantom was fabricated with meticulous attention to detail, ensuring precise dimensions to facilitate accurate simulations. The internal dimensions of the phantom measure 30 cm x 25 cm x 6 cm, while the total dimensions are 39 cm x 34 cm x 10.5 cm, including a 1.5 cm thick upper lid. This design provides a secure and controlled environment for irradiation procedures (Figure 1). The dimensions were determined considerating the maximum field size capability of the linear accelerator, which is 40 cm x 40 cm at the isocentre, and the backscatter of effect of the radiation beam.

To simulate the placement of a blood bag, a 200 mL semi-fluid-filled balloon was strategically attached inside the phantom using micropore surgical tape, so that the movement was completely restricted in CT scanning for all four media. This setup closely replicates the conditions under which the blood bag would be exposed to radiation, providing a realistic model for study. Four different medium were chosen to fill the phantom, air, water, rice powder, and thermocol, to compare their effects on radiation exposure (Figure 2).

Precise radiation dose calculations in radiotherapy treatment planning systems require an accurate correlation between computed tomography image data and electron density values. A study conducted by Damilola et al found that the radiation attenuation parameters of soy flour were not significantly different from those of water.[13] In this study, the rationale behind selecting rice powder was its electron density, which closely resembles that of water, making it an ideal candidate for simulating soft tissue. On the other hand, thermocol was chosen due to its electron density being closer to air, offering a contrast in the study of radiological properties (Table 1). The electron density of this medium were calculated from the Monaco treatment planning station using the Philips CT scanner images and the respective Hounsfield units.[14]

Four separate CT simulations were conducted using a Philips Brilliance 16 Big Bore machine with a slice thickness of 3 mm. The simulations involved each medium air (without any medium), water, rice powder, and thermocole individually, to assess the differences in radiation interaction. Fiducial markers were carefully placed on the phantom, with one positioned anteriorly and two on the lateral sides, to mark the laser origin points accurately. The images obtained from the CT scans were then transferred to the Monaco planning station for further analysis.

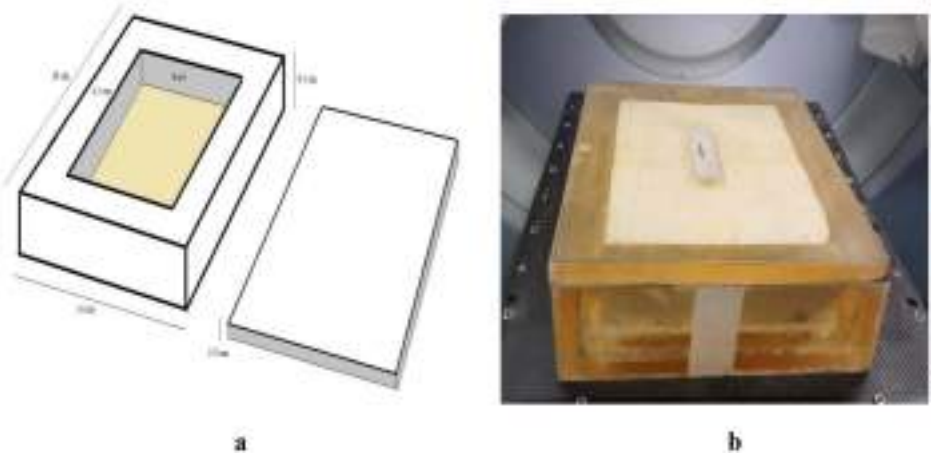

Figure 1 (**a**) Schematic diagram of blood irradiation phantom; (**b**) in-house phantom.

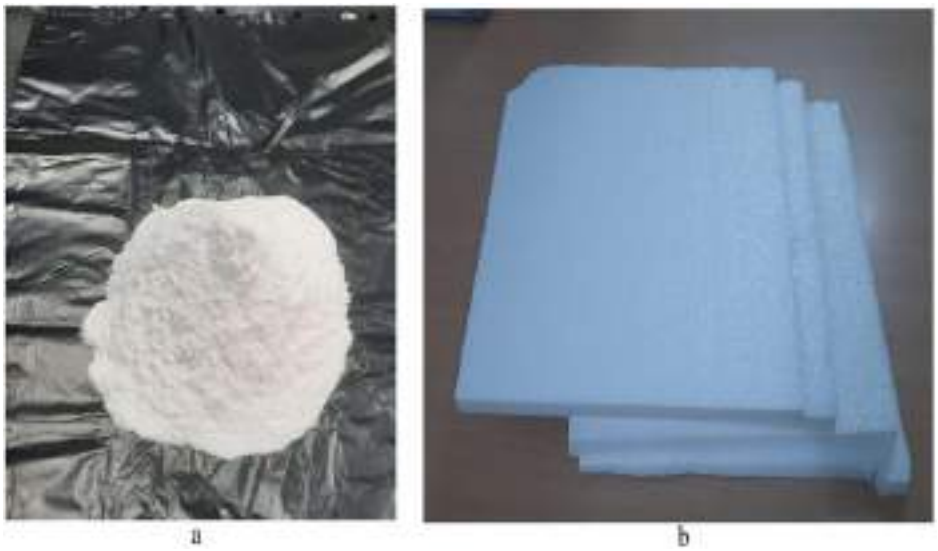

Figure 2 Material used for study: (**a**) rice powder; (**b**) thermocole.

The Monaco treatment system can be used for target delineation, registration and precise dose calculations with the help of different algorithms. In the Monaco planning station, version 5.11, the fluid-filled balloon was meticulously contoured as the target, along with separate contouring of the medium and phantom in each of the four CT images (Figure 3). Once the contouring process was complete, a 6 MV photon beam was configured in both anterior–posterior and posterior–anterior directions, ensuring the bag was positioned precisely at the isocentre in all setup medium. A field size of 35 × 35 cm² was employed using the source to axis distance technique. Calculations were performed across all media using different algorithms, such as the CC, MC, and PB, within the Monaco planning station. Each algorithm has its own pros and cons; for instance, the PB algorithm evidently did not account for the scattering geometry results in computing doses less accurately, yet it is faster than the MC algorithm.[15] Hence, a careful investigation of the accuracy of dose calculations using each beam data set and algorithm is always recommended. For each CT set, the total monitor units (MUs) for each field, along with the maximum dose (Dmax) and mean dose (Dmean) for the medium and target, were meticulously recorded using dose–volume histograms. One-way analysis of variance and Tukey's honestly significant difference (HSD) test were employed to calculate the statistical significance of p-values and assess the normality of the data for this study.

Furthermore, the study also investigated the effect of absolute dose across the different medium using a semiflex dosimeter in conjunction with the custom-built blood irradiation phantom. Field sizes of 10 x 10, 20 x 20, 30 x 30, and 40 × 40 cm² were utilized, with a source-to-skin distance of 95 cm and a monitor unit setting of 100. Meter readings were taken, and correction factors were applied to calculate the absolute dose for each field size and medium. To ensure accurate dose delivery, a RadTag® irradiation Gafchromic indicator film was employed. This film features a central dot that changes color upon irradiation, serving as a visual confirmation of radiation exposure of 25 Gy, and indicates whether the exposure was under or over the intended dose (Figure 4).

Table 1 Electron Density of Medium Used

Medium Used	Electron Density (g/cc)
Rice powder	0.93 ± 0.02
Water	1.0±0.03
Air	0.002 ± 0.002
Thermocole	0.030 ± 0.04

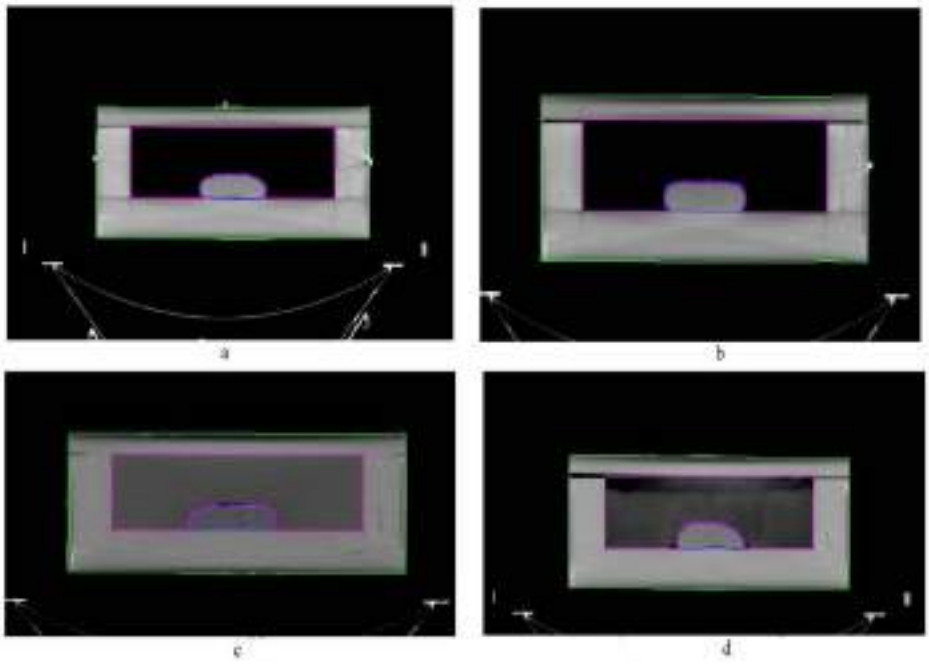

Figure 3 Axial CT view for different medium used for blood irradiation study: (**a**) air; (**b**) thermocole; (**c**) water; (**d**) rice powder.

Figure 4 Film indicator before (**a**) and after (**b**) irradiation (25 Gy).

Results and Discussion

There have been several studies dedicated to the technical aspects of the irradiation process, with minimal emphasis on the economic and managerial implications.[16–18] Petrov et al conducted a study utilizing existing equipment without requiring new investments or disrupting the clinical workflow of patients for blood irradiation. In their department, they employed rice grains as a medium to facilitate homogeneous dose distribution for blood irradiation, offering a unified procedure. Moreover, the study states that the choice of a water-equivalent rice medium satisfied the condition of a dose build-up effect.[19] However, it is noted that utilizing rice grains as a medium can leave a lot of voids; if rice powder is used instead, it will fit better and result in a more uniform dose. Although previous studies have investigated rice powder as a bolus and tissue-equivalent material, this work uniquely applies this knowledge to the domain of blood irradiation, an area where the use of rice powder has not been extensively examined In this study we were trying to evaluate the effectiveness of different materials, one of which was rice powder, which can be considered an alternate substrate for

blood irradiation. A total of four CT images with different medium (air, thermocol, rice powder and water) data were taken up for comparison. Based on the treatment planning system's dose distribution, the majority of the build-up materials were successful in producing a uniform dosage distribution inside the acrylic box except in the air medium. With rice powder, the total MU was found to be more or less the same when compared with the water medium; similar comparable dose output is measured among the air and thermocole medium. The change in monitor unit with different substrates and algorithms is shown in Figure 5 and Table 2.

The study also focused on the comparative analysis of three different dose-calculation algorithms (PB, CC, and MC) provided by the Monaco™ V 5.11 TPS, specifically in the context of blood irradiation. This comparison in the context of different substrates, including the novel use of rice powder, is an innovative approach, particularly as it also considers the impact of field size on dose distribution. The pencil beam algorithm overestimates the dose, hence MU, relative to other algorithms because of its inefficient electron spread phenomenon. The MUs calculated using the PB algorithm were less (≤2406) than those calculated by other algorithms irrespective of the medium used. Less MU (2362) is noted in thermocole material, followed by air, rice powder and water. While the PB algorithm is highly efficient in terms of time taken for computation, its limitations in handling heterogeneous media are widely recognized.[20] The data from the study show that the measured MU from the PA beam in both air and thermocole was slightly higher compared to that of the AP beam. However, in the case of water and rice powder, it is the opposite. This difference is attributed to the influence of the couch, which has a higher electron density compared to air and thermocole but is lower when compared with water and rice powder along the posterior–anterior radiation path.

It is a known fact that, in the current clinical scenario, the MC algorithm is considered to be one of the gold standards, with the highest accuracy in terms of dose computation. The MC method has shown its precision in predicting dose distribution for radiotherapy treatment planning. However, its previous drawback of long computation times has impeded its widespread use in routine clinical settings. Nevertheless, improvements in computational algorithms designed specifically for radiotherapy calculations and upgrades in computer processor technology have significantly reduced the time required for MC simulations.[21,22] Regarding the doses observed (Table 3), it was found that MC tends to produce higher maximum doses (30.26 Gy) compared to PB and CC, while PB generally yielded lower mean doses to blood when compared to the other techniques, for all materials. It was also found that, using the PB algorithm, a higher dose was noticed in air (27.84 Gy) and thermocole (27.79 Gy). This may be due to its use of a one-dimensional density correction, which failed to accurately represent the distribution of secondary electrons in various density environments.[15]

For rice powder, the highest maximum dose (30.26 Gy) was noted with Monte Carlo, which is followed by CC (26.67 Gy) and PB (27.57 Gy). Similar trends were seen for water: Monte Carlo (30.2 Gy), PB (27.55 Gy), and CC (26.3 Gy), respectively. It is well noted that the observed dose amounts for air and thermocol were similar, just like those of rice

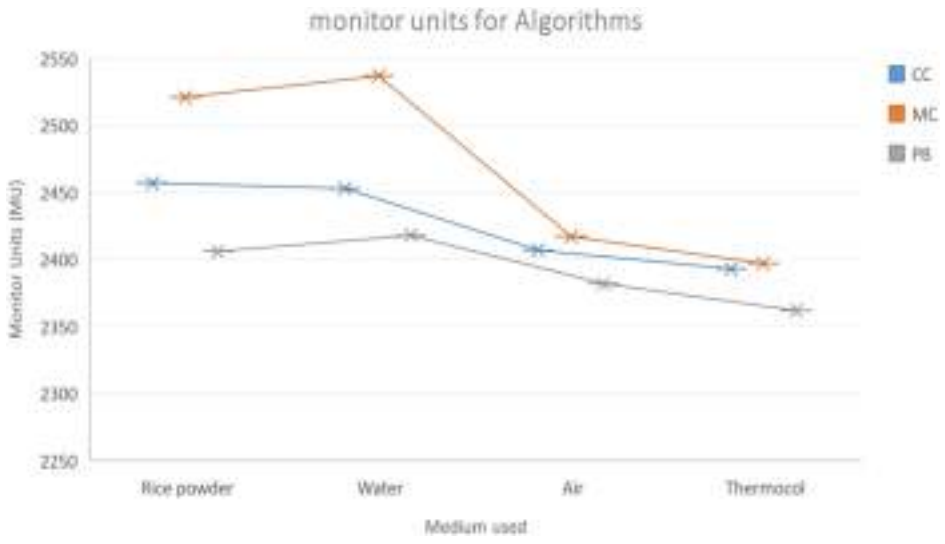

Figure 5 Impact on MU for different medium, with associated algorithms.

Table 2 Anteroposterior and Posterioanterior Beam MU for Different Algorithms and Medium

	Max Dose (Gy) (mean±SD)	Total MU (mean±SD)	PB		CC		MC		P-value (with water)
			AP	PA	AP	PA	AP	PA	
Rice powder	28.1±1.8	2461±57	1233	1173	1252	1205	1268	1253	0.99
Water	28.0±1.9	2469±61	1242	1176	1251	1201	1308	1229	0
Air	27.33±0.44	2402±18	1204	1178	1187	1220	1191	1226	0.95
Thermocole	28.0±1.2	2384±19	1183	1179	1175	1217	1187	1209	0.99

Abbreviations: Gy, Gray; SD, Standard Deviation; MU, Monitor Units, PB, Pencil Beam; CC, Collapsed Cone; MC, Monte Carlo.

Table 3 Doses Observed for Different Media and Algorithms Used

	Pencil Beam		Collapsed Cone		Monte Carlo	
	Max Dose (Gy)	Blood Mean Dose (Gy)	Max Dose (Gy)	Blood Mean Dose (Gy)	Max Dose (Gy)	Blood Mean Dose (Gy)
Rice powder	27.57	25.37	26.67	25.46	30.26	26
Water	27.55	25.48	26.3	25.42	30.2	26.13
Air	27.84	25.42	27.1	25.49	27.15	25.5
Thermocole	27.79	25.25	27	25.37	29.5	25.47

Abbreviation: Gy, Gray.

powder and water medium. The average maximum doses of all groups were assumed to be equal while using the Anova statistical test. In other words, the difference between the sample averages of all groups is not big enough to be statistically significant (p-value e0.908653). By using the Tukey HSD, there is no significant difference shown between the means of any pair shown in Figure 6. The normality assumption of the study was checked based on the Shapiro–Wilk test and all groups distributed normally.

The relative effectiveness or suitability of each medium for different field size on absolute dose was also measured and compared with the help of the semiflex chamber dosimetry system, as shown in Figure 7.

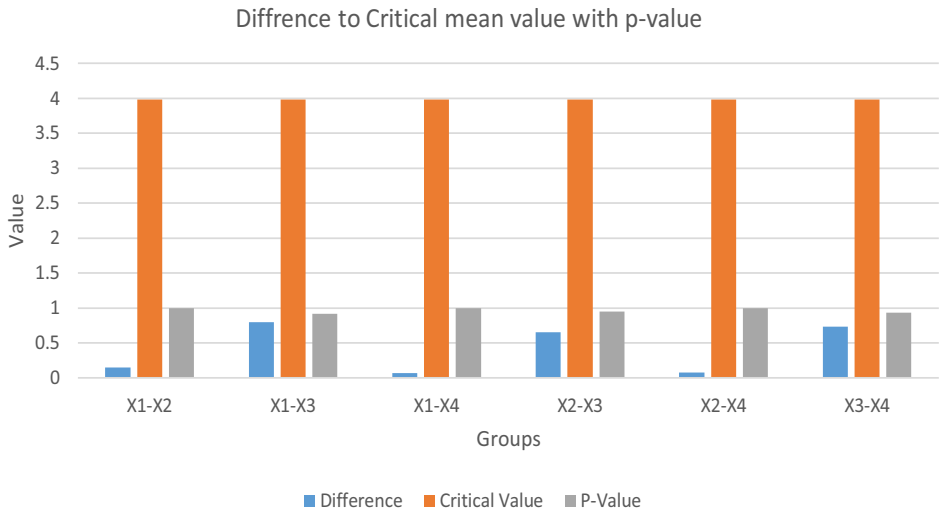

Figure 6 Statistical group-wise comparison of difference to critical mean with p-value.
Note: X1 rice powder; X2 water; X3 air; X4 thermocole.

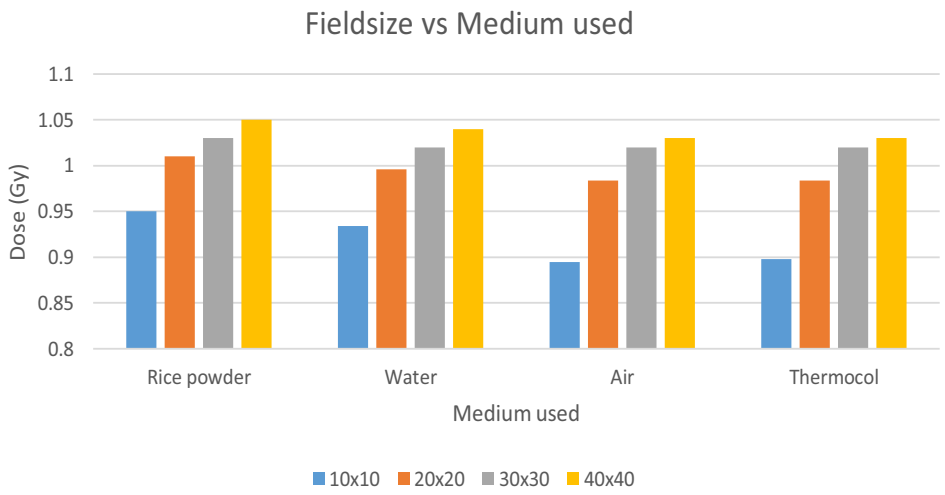

Figure 7 Impact on different field sizes using different media.

It was noted that, with increasing field size, the output dose for all medium increased proportionally.[23,24] Rice powder appeared to have consistent doses across various field sizes, with slight increases as the field size grew. Similarly, water demonstrated a comparable trend to rice powder (1.05 Gy), showing higher doses (1.04 Gy) as the field size expanded. Conversely, air indicated almost uniform doses across different field sizes, reflecting consistent dose delivery regardless of the field size. Thermocol also displayed uniform doses across various field sizes, like air. Rice powder could serve as a suitable medium for delivering scalable doses based on field size requirements, just like the water medium. In contrast, air and thermocol presented consistent doses across all fields sizes, making them more appropriate for applications where a uniform dose is needed irrespective of field size.

Increasing demand for blood irradiation in any radiotherapy department will definitely interfere with the routine treatment patient schedule. Using rice powder as a medium for blood irradiation has certain specific advantages, such as reducing setup time by up to two-thirds in comparison to water irradiation work.[4,25] Its cost-effectiveness and availability are other advantages. One of the major drawbacks with rice powder as a medium for irradiation, however, concerns practical considerations when handling and managing it, in comparison to water. Water is readily available, easy to handle, and does not require special preparation. On the other hand, rice powder needs to be properly prepared, managed, and stored to ensure consistency and safety. Another problem that may be encountered is contamination of blood products during irradiation setup. This can occur with water as well, even though the chances are very low. Contamination can be avoided by taking appropriate precautions and ensuring proper package of blood bags. All these factors should be taken into account when considering blood irradiation. The thermal property of rice powder compared to water is questionable, thus further research on this matter is advisable.

Future studies should focus on validating the dosimetric characteristics of rice powder across a broader range of radiation energies and treatment modalities to confirm its widespread applicability as a tissue-equivalent material. Examining the long-term stability and storage requirements of rice powder will be critical to ensuring its practicality in clinical settings, particularly in maintaining its dosimetric properties over time. Further expanding the investigation to include comparisons with other potential alternative media could identify the most effective substitute for water, providing a comprehensive evaluation of available options.

Conclusion

In this research, dosage computation for blood irradiation using PB, CC, and MC algorithms was quantitatively examined using various medium such as air, water, rice powder, and thermocol. The total monitor units for both rice powder and water were found to be nearly equivalent across all three algorithms and different field sizes. When comparing thermocol with other medium, its response closely matched that of air. It was observed that fewer monitor units were recorded in PB

while more monitor units were noted in the MC algorithm regardless of the medium used. Additionally, the maximum dose was found to be higher when using the MC algorithm, especially using rice powder.

It has been observed that utilizing rice powder as a medium for blood irradiation offers notable advantages in terms of cost-effectiveness and availability of resources, as well as minimizing or no spillage onto machine components. Additionally, the setup time for blood irradiation is reduced when compared with the water medium. Consequently, it can be concluded that rice powder, due to its similarity in electron density to water, presents a viable alternative medium for blood irradiation.

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Disclosure

Sarath S Nair is the primary author. The authors have no relevant conflicts of interest to disclose for the present study.

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Evaluation of IgG and Complement Component C4 Levels in Low-Income Countries, Yemen Republic in Light of Their Proposed Role in the Hemolysis of Stored CPDA-1 Whole Blood

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Evaluation of IgG and Complement Component C4 Levels in Low-Income Countries, Yemen Republic in Light of Their Proposed Role in the Hemolysis of Stored CPDA-1 Whole Blood

Jamil MAS Obaid[1,2], Khawla AAS Sakran[2], Shaima AH Mohammed[1], Shifa`a LA Al-Salahi[1], Nawal AN Mahdi[1], Mohammed AM AL-Sharabi[1], Asadaddin SM AL-Gaadi[2], Mohammed NM AL-Fatahi[2]

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Objective: Hemolysis is the most severe change that occurs in stored blood and can cause severe consequences in patients after transfusion. This study examines the potential role of IgG and complement, exampled by C4, in the hemolysis of stored CPDA-1 blood under poor storage conditions in low-income countries.

Methods: The study was performed on 30 whole blood units (250 mL) drawn from convenience healthy volunteer donors with CPDA-1 anticoagulant and stored at 2–6 °C for 35 days. Each well-mixed blood bag was sampled at 0, 7, 21 and 35 days and examined for CBC, plasma hemoglobin, hemolysis percent and determination of IgG and C4.

Results: The plasma hemoglobin level and hemolysis percent increased continuously to reach 1.56 g/dl and 7.05% at the end of storage time. Hemolysis increased alongside the mean IgG concentration that was increased significantly from day 0 of storage (7.68±1.75 g/L) and peaked on day 7 (11.55±1.57 g/L), then declined to reach 8.33±2.09 g/L on day 35. Also, the mean concentration of C4 increased from day 0 of storage (0.15±0.06 g/L) to a peaked on day 21 (0.18±0.04) then declined on day 35 (0.17±0.06 g/L). The coordinated action of IgG and C4 is reflected by the positive correlation of their delta changes ($r=0.616$, $p<0.0001$).

Conclusion: Elevated hemolysis percent in whole CPDA-1 stored blood in Yemen was accompanied by initial increase of IgG and C4 followed by final decline, which indicate their activation and consumption during hemolysis. Further studies for other hemolysis markers and analyses will give a full idea about that.

Keywords: IgG, complements, hemolysis, CPDA-1 blood, transfusion

Introduction

Transfusion represents a suitable therapy for the treatment of critically ill anemic patients. Whole blood transfusion for anemic patients is still the first type of blood product used in most developing countries, including Yemen, which has been in war for 9 years.[1] Despite its essential use in the treatment of some situations, such as acute blood loss in accidents and shots, the use of whole blood in other cases contributes to meeting the need for blood. Some data suggest that the gap between need and supply in low-income and middle-income countries is large, and the WHO also targets 10–20 donations per 1000 people for many countries.[2] A study of blood transfusion among pediatric patients in Aden city, Yemen, reported a frequency of 26.4% for whole blood transfusions.[3] Blood transfusion in Yemen is carried out with a little improvement, most clinical transfusion therapy uses whole blood, and little components are produced (only packed RBCs and platelet-rich plasma) at a narrow range.

During storage, many changes affect many components and aspects of blood. Preservatives such as citrate phosphate dextrose adenine (CPDA-1) were used to ameliorate these changes and extend the lifespan of red blood cells (RBCs).[4]

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Changes in RBCs may be attributed to the storage conditions (mainly a storage temperature that must be ranging from 2–6 °C) and donor attributes, which are reflected in donated blood.[5,6] The previously reported causes of changes in stored blood are metabolite accumulation or depletion and oxidative damage that eventually leads to increased red cell hemolysis.[7] Most biochemical and cellular changes include increased intracellular potassium, increased cytoplasmic sodium, decreased glucose, decreased pH, changes in RBC morphology and membrane protein composition, and the production of harmful membrane fragments, microparticles, and free hemoglobin.[8,9] However, the effects of blood storage time and the extent to which stored blood is safe and potent remain the main issues of debate. Prolonged blood storage increases mortality, serious infections, and multiorgan failure after transfusion.[10]

RBC hemolysis in stored blood bags occurs during blood collection, processing, handling and storage. RBC hemolysis above the accepted level (1% according to international guidelines) is a marker of RBC storage failure.[11] RBCs are very fragile as they age, and consequently, cell-free hemoglobin and microparticles are released. Cell-free hemoglobin and microparticles lead to further hemolysis and breakdown of RBCs.[12] Leukocytes contribute to hemolysis through the release of various chemicals and enzymes, especially proteases. Therefore, the use of leukocyte reduction filters decreases the hemolysis rate in stored blood.[13]

The induction of immunological mechanisms may be indicated by many marker changes in stored blood that can be initiated by antibody sensitization of red blood cells followed by complement activation via the classical pathway. For instance, it was reported that leukocyte-reduction filtration decreases the hemolysis rate.[14] Thielen et al, in their in vitro study, showed that RBCs in stored blood were sensitized by antibodies and complement independent of storage time.[15] In vivo, leukocytes can increase humoral responses activated by T-helper-2.[16] This study examines the potential role of IgG and complement components (C4, a prototype component of the classical activation pathway) in the hemolysis of stored CPDA-1 blood under poor storage conditions in low-income countries.

Methods

Overview of the Study

This prospective experimental study was conducted at the Faculty of Medicine and Health Sciences-Ibb University, Yemen, for the period from May 31, 2023, to July 5, 2023. The study was performed on whole blood units (250 mL) that were drawn from suitable healthy volunteer donors into a CPDA-1 anticoagulant-containing single blood bag and stored to the expired date after 35 days.

A convenience sample was used to select participants. After checking the donor eligibility requirements, 30 donors were selected; 7 of them were females, and 23 were males with all ABO phenotypes. The donors were aged between 18 and 33 years. Informed consent was obtained from each healthy volunteer verbally. This study was conducted according to the international ethical guidelines of medical research, mainly the Declaration of Helsinki–ethical principles for medical research involving human subjects, in 2013.

The inclusion criteria included healthy donors who met the whole blood donation eligibility requirements, who were aged at least 18 years, who had a normal hemoglobin level, and who were free from disease for at least 6 months. The exclusion criterion for all donors was that they did not meet the eligibility requirements of the American Association of Blood Banks (AABB).

Experimental Design

A total of thirty healthy volunteer donors had the following ABO phenotypes: 9 with the A blood phenotype, 3 with the B phenotype, 7 with the AB phenotype, and 11 with the O phenotype. Eligible donors underwent venous blood collection for donation, with appropriate care and adequate safety precautions to prevent donor and worker infections and to avoid contamination of blood units. A total of 250 mL of blood was drawn from each volunteer donor in a single blood bag with approximately 35 mL of CPDA-1 as an anticoagulant. The blood was gently mixed during collection, immediately placed in a disinfected blood bank refrigerator and kept at 2–6 °C for 5 weeks (35 days).

During storage, approximately 10 mL of each well-mixed blood bag was collected aseptically at 0, 7, 21 and 35 days of storage. Each sample was analyzed for complete blood count (CBC) and then centrifuged for 10 min at 4000 rpm to

obtain plasma. The plasma was used for the measurement of free plasma hemoglobin spectrophotometrically and, consequently, for the calculation of hemolysis percent. Part of the separated plasma was kept in an Eppendorf tube and immediately frozen at –20 °C for the analysis of IgG and complement C4.

ABO and Rh Blood Grouping

The ABO blood group of the donors was determined by direct agglutination testing using the anti-sera, anti-A, and anti-B reagents (Agappe, India).

Complete Blood Count

CBCs were analyzed for each sample on an automatic hematology analyzer (Sysmex XS-500i, Japan). A Sysmex XS-500i hematology analyzer was used for flow cytometry to analyze the physiological and chemical properties of the cells. All parameters of red blood cells, white blood cells and thrombocytes were obtained and reported properly on each examination day.

Plasma Hemoglobin Determination

The plasma Hb concentration was measured using the cyanmethemoglobin method by mixing 20 µL of plasma with 5 mL of Drabkin's reagent (Hemoglobin Monlab test, Spain), and the absorbance was measured after 5 minutes spectrophotometrically at 540 nm (BTS–350, Biosystems, Spain).

Calculation of Hemolysis Percent

The percentage of hemolysis in the stored whole blood unit was calculated according to the following formula:

$$\text{Hemolysis\%} = \text{Plasma hemoglobin}\left(\text{gdl}^{-1}\right) \times (1 - \text{Hct}) \times 100/\text{Total hemoglobin}\left(\text{gdl}^{-1}\right)$$

Immunoglobulin G and C4 Determination

The plasma samples were analyzed for IgG and C4 with an immunoturbidimetric assay technique using the IGG-2 CAN 674 and C4-2 Tina-quant kits (Roche Diagnostics, Germany) and an immunological analyzer Cobas c311 (Roche/Hitachi, Japan) at a wavelength of 340 nm.

Statistical Analysis

The data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) version 19. The data were analyzed with descriptive statistics to determine the means, SDs, frequencies, and percentages, as appropriate. A *t* test or Mann–Whitney rank sum test was used to analyze the difference between two groups, or ANOVA was used for more groups. Spearman correlation tests were used for correlation analyses. A p value ≤ 0.05 indicated statistical significance.

Results

The most important change in RBC parameters was an increase in the mean cell volume (MCV) from day 0 to the end of storage (from 81.52±5.29 fl to 87.70±6.08 fl, p=0.001). The red cell distribution width (RDW) also increased (from 12.66 ±1.13 to 14.97±1.18, p<0.0001). Moreover, the mean cell hemoglobin concentration (MCHC) decreased from 36.72±0.88 g/dl to 33.76±0.97 g/dl. (Table 1). WBCs showed a significant reduction in total count or as an individual absolute leukocyte type, except for lymphocytes, which increased in number with increasing incubation time. The mean thrombocyte count generally increased significantly from 177±39.65*10^9 PLTs/L to 290±113.87*10^9 PLTs/L during storage. Other parameters, such as the mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR), also showed gradual increases compared with the baseline values on day 0. All these data are listed in Table 1.

The changes in the mean plasma hemoglobin level and the mean hemolysis percent of the stored blood are depicted in Figure 1. Both showed continuous elevation, with a statistically significant difference between measurements (p<0.0001). The continuous increase in these parameters was also significantly different among the different ABO phenotypes, except for B blood group patients, as listed in Table 2.

Table 1 Changes in the Mean CBC Parameters of the Stored CPDA-1 Whole Blood Samples During Storage

Parameter	Day 1 Mean ± SD	Day 7 Mean ± SD	Day 21 Mean ± SD	Day 35 Mean ± SD	*P* value
Red Blood cells parameters					
Hb (g/dl)	13.51±1.52	14.03±2.54	14.24±2.63	13.47±1.56	0.401
RBCs (*10^{12}\l)	4.53±0.53	4.77±0.84	4.74±0.84	4.56±0.56	0.431
PCV (%)	36.76±3.73	39.72±6.62	40.17±7.26	39.87±4.55	0.079
MCV (fl)	81.52±5.29	83.65±5.62	84.83±5.81	87.70±6.08	0.001
MCH (pg)	29.96±2.33	29.75±2.84	30.05±2.36	29.62±2.32	0.904
MCHC (g/dl)	36.72±0.88	35.19±0.74	35.40±0.73	33.76±0.97	0.0001
RDW (%)	12.66±1.13	13.43±1.0	14.09±1.02	14.97±1.18	0.0001
White Blood cells parameters					
Total WBCs (*10^9\l)	5.30±1.51	5.48±1.53	4.31±1.01	4.65±1.18	0.002
Neutrophils (*10^9\l)	2.48±1.19	2.50±0.89	2.10±0.72	1.97±0.84	0.060
Lymphocytes (*10^9\l)	1.94±0.59	2.07±0.49	2.0±0.54	2.36±0.94	0.079
Monocytes (*10^9\l)	0.43±0.14	0.68±0.48	0.12±0.10	0.10±0.10	0.0001
Eosinophils (*10^9\l)	0.43±0.31	0.21±0.22	0.06±0.05	0.17±0.13	0.0001
Basophils (*10^9\l)	0.02±0.02	0.03±0.02	0.02±0.03	0.06±0.04	0.0001
Platelets parameters					
Platelets (*10^9\l)	177±39.65	223±67.75	190±48.21	290±113.87	0.0001
MPV (fl)	8.07±0.57	8.64±0.58	8.84±0.52	8.65±0.70	0.0001
PCT (%)	0.14±0.03	0.19±0.07	0.17±0.05	0.25±0.10	0.0001
PDW (fl)	14.70±1.14	16.22±1.60	16.29±2.0	15.22±1.21	0.0001
P-LCR (%)	15.36±4.60	19.37±4.82	22.03±4.07	20.44±4.93	0.0001

Abbreviations: Hb, hemoglobin; RBC, red blood cells; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; WBCs, white blood cells; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; P-LCR, platelet large cell ratio.

The mean immunoglobulin G level increased significantly from day 0 of storage (7.68±1.75 g/L) to day 7 (11.55±1.57 g/L) and then declined to 8.33±2.09 g/L on day 35, as illustrated in Figure 2. However, the mean complement C4 level increased insignificantly from day 0 of storage (0.15±0.06 g/L) to peak on day 21 (0.18±0.04) and declined on day 35 (0.17±0.06 g/L), as

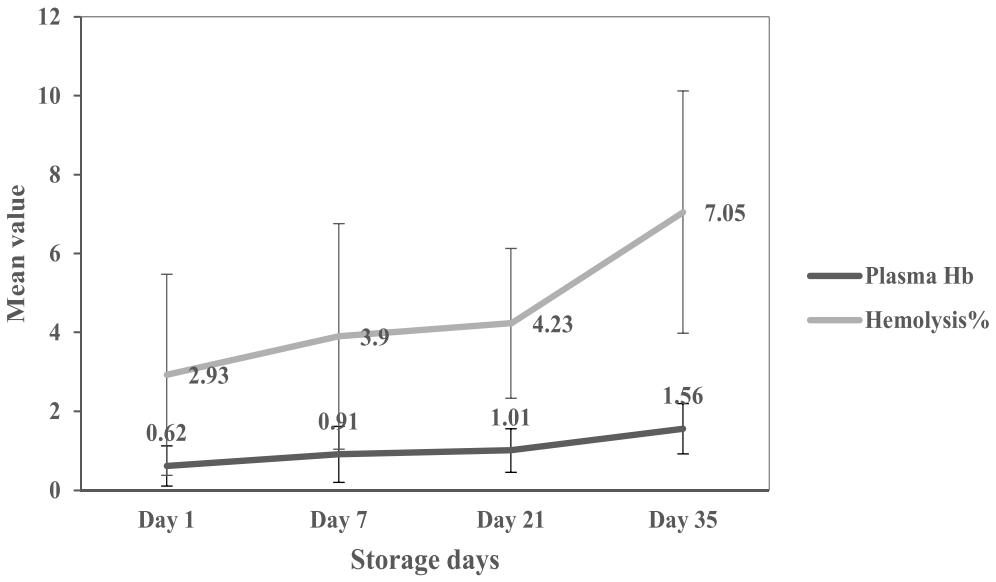

Figure 1 Changes in the mean plasma hemoglobin level and the mean hemolysis percent of the stored blood during storage.

Table 2 Changes in the Mean Plasma Hemoglobin Concentration and Hemolysis Percent of Donated Blood from Different ABO Blood Group Phenotypes During Storage

Blood Group	Parameter	Day 1 Mean ± SD	Day 7 Mean ± SD	Day 21 Mean ± SD	Day 35 Mean ± SD	*P* value
A	Plasma Hb	0.70±0.47	0.83±0.43	0.92±0.45	1.34±0.54	0.040
	Hemolysis%	3.27±2.02	3.73±2.39	4.08±2.07	6.03±2.12	0.052
B	Plasma Hb	1.22±0.70	0.33±0.26	1.02±0.18	1.70±1.03	0.148
	Hemolysis %	6.0±4.07	1.53±1.36	4.57±0.50	8.20±5.83	0.229
AB	Plasma Hb	0.38±0.23	0.80±0.44	1.10±0.64	1.91±0.55	0.0001
	Hemolysis %	1.61±1.05	3.23±1.89	4.90±2.48	7.79±2.15	0.0001
O	Plasma Hb	0.59±0.54	1.22±0.99	1.03±0.68	1.48±0.65	0.044
	Hemolysis %	2.82±2.63	5.11±3.62	3.84±1.68	7.09±3.53	0.011

Abbreviations: Hb, hemoglobin; SD, standard deviation.

depicted in Figure 3. The delta changes in IgG levels moderately correlated with the delta changes in C4 levels (r=0.616, p<0.0001), as shown in Figure 4.

The plasma hemoglobin and hemolysis percent which are strongly correlated (r= 0.954, p<0.0001), were positively correlated with the traditional affected variable MCV and platelet count and negatively correlated with the MCHC and absolute monocyte count (Figure 5). The IgG level was negatively correlated with MCH, MCHC and eosinophils but positively correlated with platelet count. Similarly, C4 was negatively correlated with MCH, MCHC and the absolute neutrophil count but positively correlated with the absolute lymphocyte count (Figure 6).

Discussion

Blood for transfusion must be kept at optimum storage conditions to achieve good posttransfusion survival of cells to fulfill their desired functions. The cellular and chemical changes that occur in stored blood have been studied and have yielded some recommendations for improving storage conditions. This study is a contribution in this field and assumes a potential role for IgG and complement proteins in the hemolysis of stored blood, taking into consideration the situation in low-income countries with limited resources, such as the Yemen Republic. One of these considerations is the transfusion of whole blood for most patients who must receive one blood component rather than whole blood because of the poor service of blood banks and the lack of double, triple and quadruple blood bags. Therefore, the preparation of

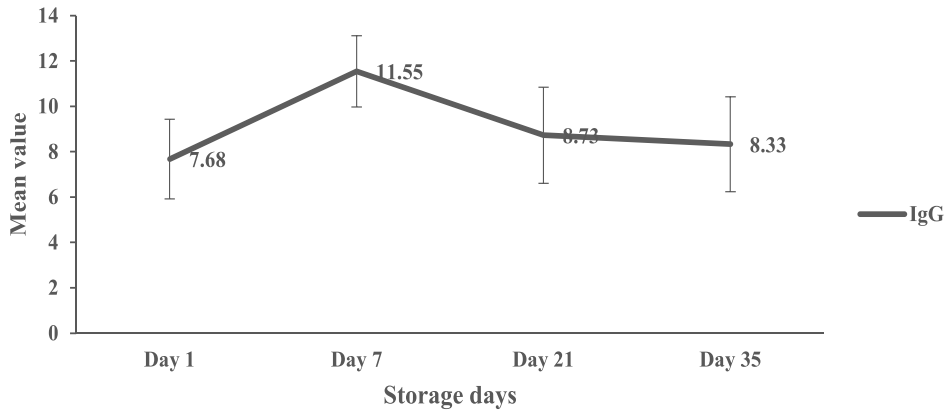

Figure 2 Changes in the mean IgG level in the stored blood during storage.

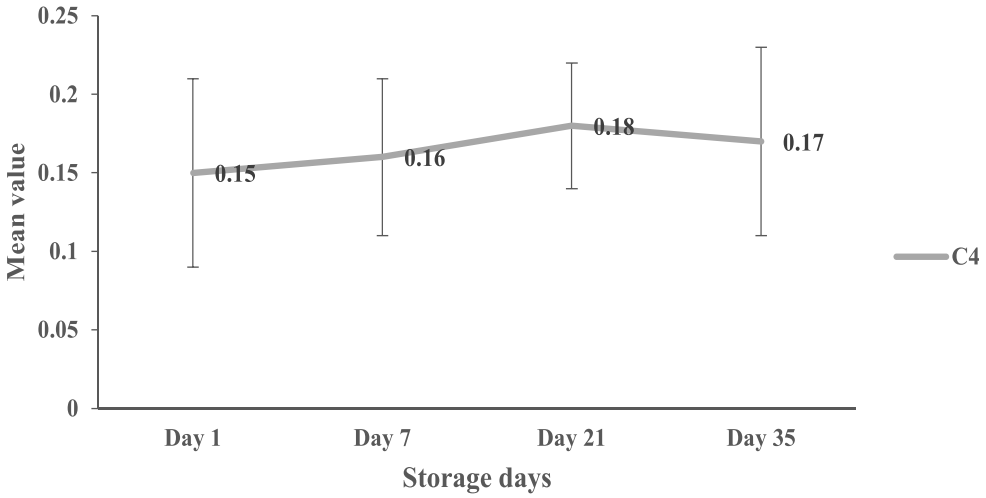

Figure 3 Mean C4 level change in the stored blood during the storage time.

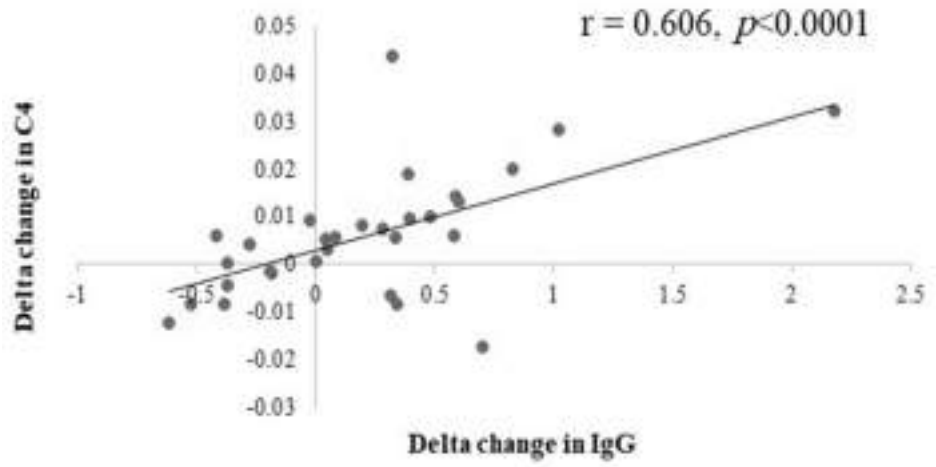

Figure 4 Correlation analysis between delta change in IgG level and delta change in complement component C4 of CPDA-1 stored blood.

blood components is unattainable. Similarly, whole blood transfusions are also performed in many centers in another low-income country, Nigeria.[17]

Blood storage in vitro causes cellular changes resulting in reduced red blood cell survival that may culminate in hemolysis. The accumulation of changes with increasing storage time was studied for the extent to which red blood cells lost their safety and efficacy and for the increased risk of transfusion complications when transfused blood was stored for long periods even before the expiry date.[18,19] Stored red blood cells undergo hemolysis and microparticle formation, which is an ever-dangerous change. Moreover, hemolysis increased significantly over time in the stored blood,[20] which is in accordance with our results showing that hemolysis changes progresses during storage. Hemoglobin in microparticles that is released from hemolysis may represent a common factor driving multiple pathways leading to negative consequences; these pathways include oxidative stress, nitric oxide depletion and platelet activation.[21]

An increase in the MCV and RDW and a logic decrease in the MCHC are the traditional significant changes in RBC parameters reported here as well as in both previous and recent studies.[22–24] The total WBC count, absolute monocyte and eosinophil counts were decreased, in agreement with the findings of a previous study that attributed this decrease to the degeneration of leukocytes.[25] An increase in the mean absolute lymphocyte count may be due to an artifactual cause, where the leukocytes prepared for apoptosis condense their nucleus and decrease in size, which may give these cells

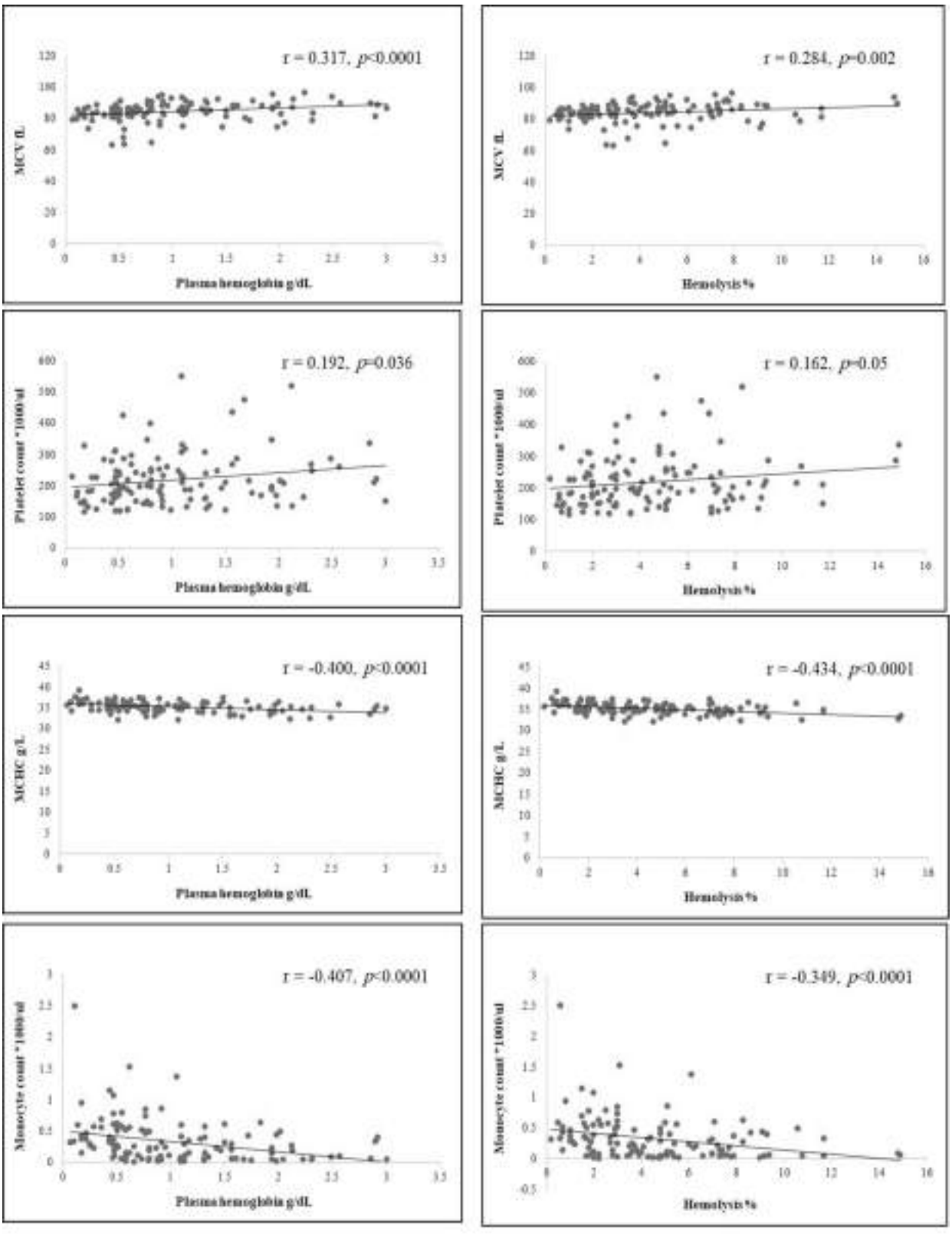

Figure 5 Correlation analysis between plasma hemoglobin, hemolysis percent with MCV, platelet count, MCHC and absolute monocyte count of CPDA-1-stored blood.

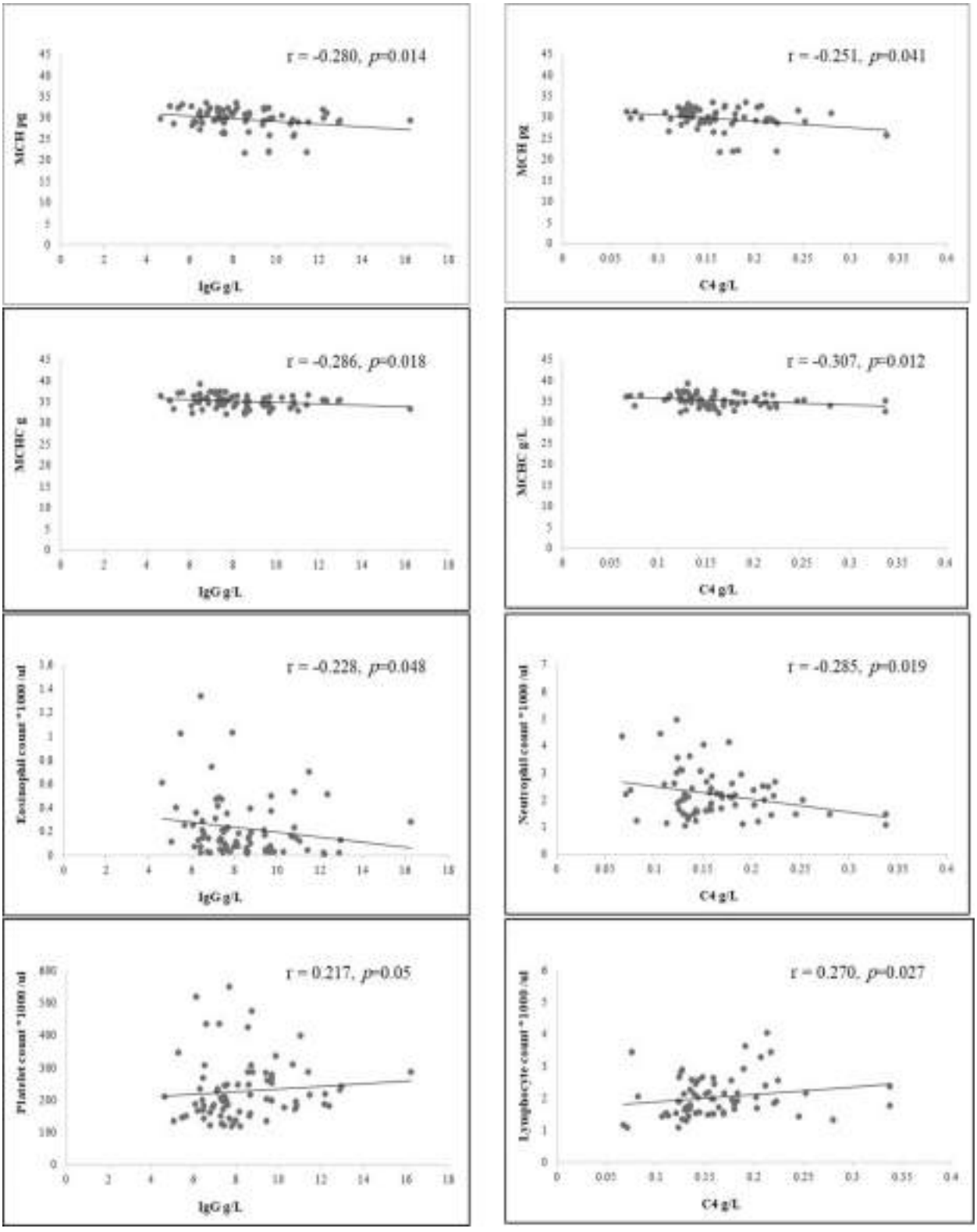

Figure 6 Correlation analysis between the IgG and complement component C4 levels and the MCH, MCHC, absolute monocyte, eosinophil and lymphocyte counts in CPDA-1-stored blood.

a small lymphocyte appearance; hence, they may be counted as lymphocytes. Surprising changes were observed in the platelet count and indices. An increase in the PLT is artifactual and is attributed to an increase in the number of microvesicles or microparticles released from RBCs and WBC fragmentation during storage. Because of their similar

sizes, fragments may be counted as platelets by a hematology analyzer. Another possibility is the reversal of the aggregation process that may occur for platelets under warming conditions during sampling.[26]

As mentioned previously, hemolysis is the worst manifestation of red cell lesions during storage, and the most important marker determines the suitability of blood for transfusion. The parallel increase in plasma hemoglobin and hemolysis percent with advance storage time was predicted to occur as a result of red cell storage damage. This was also reported in this study, as well as in previous studies. It is the greatest change occurred during the last week of storage.[26–28] Hemolysis was not associated with a certain ABO phenotype despite the lack of a statistically significant difference in the changes in plasma hemoglobin and hemolysis percent among B blood group donors, which may be attributed to the small sample size. Both markers were positively correlated with the conventional marker MCV and platelet count and inversely correlated with the MCHC. Plasma hemoglobin and hemolysis percent were associated by a moderate negative correlation with the absolute monocyte count, this may refer to monocyte consumption in phagocytosis activity.

The high hemolysis rate in this study—above the internationally accepted limits, FDA accepted level is 1% —is related to poor storage conditions, including an unstable electrical power supply for refrigerators and deterioration of the collection bags quality due to difficulties of their importing and transporting processes in low-income countries. In terms of the biological mechanisms responsible for hemolysis, leukocytes contribute significantly to hemolysis via several pathways. Leukocyte release of various chemicals and enzymes, especially proteases that cause red cell lysis, using leukocyte reduction filters decreases the rate of this hemolysis in stored blood units.[27] Unfortunately, leukoreduction is rarely performed during blood transfusion in Yemen.

This study showed a gradual increased IgG level and complement C4 components during storage. Increased IgG level with storage in non-leukoreduced blood was also proved in agreement with our result by Antonelou et al,[29] Therefore, these findings lead our hypothesis that suggest IgG and complement contribution in hemolysis of the stored blood by an immune mechanism start by IgG binding to RBCs and consequent complement activation via classical pathway. This mechanism culminates in red cell lysis. Storage causes continuous decrease of red cell hemoglobin content (decrease MCHC) and its precipitation on red cell that may alter the membrane leading to red cell sensitization by IgG and complement activation. Thus, this study showed a negative correlation between IgG and C4 levels and MCHC. The deposition of IgG and complement component C3 on stored RBCs has been proven previously, despite the limited uptake and phagocytosis by macrophages in vitro, according to Thielen et al.[15] Meanwhile, Hult et al proved that in vitro phagocytosis of red blood cells can occur in the presence of serum.[30] Therefore, the presence of serum will activate macrophage as the situation in this study. IgG binding and complement activation on RBCs develop because of membrane alterations of the stored red cells was proved previously.[31] An important supportive results are the manner by which IgG and complement changed. The reasonable and coordinated suggested scenario is the gradual increase of IgG firstly peaked on day 7 to sensitize red cells, this is followed also by a gradual increase in C4 component peaked on day 21, both of which were declined later. The final decline in these markers denotes their consumption after activation. The coordinated increase in both markers was evidenced by the direct correlation between the delta change in the IgG level and the delta change in complement component C4 ($r=0.661$, $p<0.0001$).

Antibody-dependent cell-mediated cytotoxicity and opsonization mechanisms can also be suggested. The supportive results were the negative correlations between C4 and neutrophil count, between IgG and eosinophil count, and between plasma hemoglobin and hemolysis percent with monocyte count. Phagocytosis of sensitized cells by antibodies and complement may also lead to phagocyte cell exhaustion and apoptosis, as proposed by Frankenberg et al.[32]

The limitation of this study lies in the lack of financial support to explore additional markers of hemolysis in relation to the supposed immune mechanisms studied in this paper. Additional analyses are needed to explore certain immune mechanisms in the hemolysis of stored blood using more markers and advanced techniques. Furthermore, the findings of this in vitro study cannot be exactly translated to what we would expect in vivo without performing clinical trial studies. It is recommended to generalize leukoreduction strategy in all transfusion centers, also transfuse blood units as early as possible before the expiry date to avoid the bad effects of storage.

In conclusion, the higher hemolysis percent in whole CPDA-1 stored blood in Yemen, a low-income country, warrants further investigations for implicated mechanisms and prevention strategies. The immunological mechanism of hemolysis mediated by IgG and the ensued complement activation via the classical pathway in the whole CPDA-1 stored blood is strongly suggestive in this study and pronounced by their final decrease (due to consumption) after initial

increase (due to activation). Plasma presence and non-leukodepletion in this blood units also enhance the phagocytic activity of macrophages in blood units. Further studies for other hemolysis indicators and analyses will provide us with a full idea about that.

Abbreviations

CBC, complete blood count; IgG, immunoglobulin; CPDA-1, citrate phosphate dextrose adenine; AABB, American Association of Blood Banks; SPSS, Statistical Package for Social Sciences; ANOVA, analysis of variance; MCV, mean cell volume; RDW, red cell distribution width; MCHC, mean cell hemoglobin concentration; MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet large cell ratio, PLTs; platelets.

Data Sharing Statement

All data are available in this manuscript.

Ethical issue

This study was conducted under the approval of ethical committee at the Medical Laboratory Sciences Department at the Faculty of Medicine and Health Sciences, Ibb University (Ethical approval No. 13-MLS-Feb. 2023). An informed consent for all donors was taken verbally with authorization from Ethical Committee because all donors were students at our faculty and belong to our scientific community, verbal consent in this community is sufficient. They are in daily contact with the ethical committee. A third-party witness, the faculty's general manager, was present during the donation procedure and told the committee. All research processes also committed to the international ethical guidelines of medical research, mainly the Declaration of Helsinki–ethical principles for medical research involving human subjects, in 2013.

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Disclosure

The authors have disclosed no conflicts of interest.

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Paving the Road for Haematopoietic Stem Cell Transplantation in the United Arab Emirates: A Single Centre's Experience

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Open Access Full Text Article SHORT REPORT

Paving the Road for Haematopoietic Stem Cell Transplantation in the United Arab Emirates: A Single Centre's Experience

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Background: Despite its long-term history, haematopoietic stem cell transplantation (HSCT) still faces challenges in several countries under development and especially in the private health sector.

Objectives, Design and Methods: In this retrospective analysis, we present our experience and the results of 48 adult patients who underwent HSCT (autologous 37, allogeneic 9) at a private-sector hospital in Abu Dhabi, United Arab Emirates (UAE). The main indications were multiple myeloma and acute myeloid leukaemia in the autologous and allogeneic setting, respectively.

Results: All patients successfully engrafted, and after a median follow-up of 6 (range: 1–11) months, 42 patients are alive (36 autografted and 6 allografted). The 1-year overall survival rates were 97% and 62% for autografted and allografted patients, respectively, while 4 patients died within 100 days post-transplant from treatment-related causes (1 patient from the autografted group and 3 patients from the allografted group).

Conclusion: Our data confirm that HSCT is a feasible, safe, and effective treatment approach, offering high success rates to UAE patients with haematological malignant diseases.

Keywords: autologous stem cell transplantation, allogeneic stem cell transplantation, middle east, development, barriers, United Arab Emirates

Haematopoietic stem cell transplantation (HSCT) is considered the standard of care for patients with otherwise incurable but chemo- and immune-sensitive malignant and non-malignant disorders.[1] Despite its complexity and the need for specialized centers with contemporary and high levels of lab support along with highly experienced medical staff, HSCT is currently a routine in Western countries; however, it is still "travelling the initial steps" in several Eastern countries, including the United Arab Emirates (UAE).[2] Until recently, and despite its fast-growing economy, the UAE did not have a local health programme for patients who need HSCT. Therefore, several patients seek HSCT abroad every year, thus resulting not only in high expenditure for the procedure itself but also in suboptimal post-transplant monitoring due to the lack of centers with experienced medical staff and proper laboratory support.[3]

In February 2021, we initiated the establishment of the first comprehensive adult and paediatric HSCT unit in the UAE, in Burjeel Medical City (BMC) Hospital. Physicians and nurses, having prolonged experience in haematopoietic stem cell transplantation in international centers (Europe, USA, India), staffed the HSCT unit in BMC. Nowadays, the

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ultimate outcome in HSCT procedures is broadly dependent on the laboratory support. In BMC Hospital, apart from the standard laboratory tests, more specific tests like flow cytometry assays or tests of minimal residual disease (MRD) are largely provided as in-house tests. For those not provided in-house, there is a send-out service offering timely the requested results.

In October 2021, we performed the 1st autologous and, in September 2022, the 1st allogeneic HSCT in adult patients.[3]

We herein report our experience and outcomes of the first 48 adult HSCT performed during the last two years in the private sector in the UAE. This study complies with the Declaration of Helsinki, and approval from the institutional Ethics Committee has been obtained.

From October 2021 till September 2023, 39 autologous and 9 allogeneic transplants were carried out in our HSCT unit, including 27 males and 21 females with a median age of 45.5 years (range: 18–67). Multiple myeloma was the main indication for autoHSCT and acute myeloid leukaemia for alloHSCT (detailed patient characteristics are summarized in Table 1). The conditioning regimens were chosen based on the primary disease, the patient's age, and the existence of co-morbidities. Forty-four patients received myeloablative regimens (standard BEAM, high dose Melphalan, TBI-Cy, Thiotepa 10 mg/kg – Busulfan 9.6 mg/kg – Fludarabine 150 mg/m^2, or Busulfan 12.8 mg/kg – FLU 120 mg/m^2), while 4 allografted patients received a reduced intensity regimen (Thiotepa 5 mg/kg – Busulfan 6.4 mg/kg -Fludarabine 150mg/m^2 or Busulfan 9.6 mg/kg – FLU 90 mg/m^2), either due to their age or because of co-existing comorbidities. In the autologous setting, the haematopoietic stem cells (HSCs) were mobilized either with chemotherapy plus granulocyte colony stimulating factor (GCSF) or GCSF alone; plerixafor on demand was used in 10 patients. The HSCs for 9 allografted patients were collected from either full-matched siblings (n=6) or haploidentical donors (n=3) after the use of GCSF only (Table 1). All allografted patients received immunosuppressive prophylaxis for acute graft vs. host disease (aGvHD), related to the donor type and the conditioning regimen intensity (Table 1).

The statistical analysis was performed by SPSS version 22. The Kaplan–Meier method was used to calculate the differences and the survival rates.

All individuals (patients and healthy donors) successfully mobilized, and the 39 autografted patients and the 9 allografted patients received a median of 4.3 x 10^6/kg (range: 2.2–21.4) and 6 x 10^6/kg (range: 4.4–8.2) CD34+ cells, respectively. Engraftment was prompt and successful for all patients, at a median of 12 days (range: 9–33) post graft infusion (Table 1).

Among allografted patients, all but one achieved full donor chimerism within a median of 30 days (range 20–103). One patient with diffuse large B-cell lymphoma, refractory to multiple lines of treatment, received escalating doses of donor lymphocyte infusions (DLIs) because of residual disease with borderline mixed haematopoietic chimerism (94–95% of donor origin), and the patient is currently alive with residual disease after additional chemotherapy. Clinically significant aGvHD developed in 3 patients, while chronic GvHD occurred in 2 out of 5 evaluable patients (Table 1).

After a median follow-up of 9.6 months (range: 1.2–24), 44 patients (38 autografted and 6 allografted) are alive, and 36 of them (31 autografted and 5 allografted) are assessed with undetectable disease. For the autografted patients, the projected 2-year overall survival (OS) and the 2-year progression-free survival (PFS) were 97% and 81%, respectively (Figure 1).

Importantly, all patients who were allografted with undetectable disease at the time of transplant are alive and progression-free, with a median follow-up of 5 months (range: 2–9) post-transplant. On the contrary, for patients who were allografted with active disease, the median OS and median PFS were only 2 months. Four out of 48 patients died from treatment-related causes; 1 out of 39 autografted patients died from massive central nervous system bleeding, and 3 out of 9 allografted patients died from steroid-refractory aGvHD grade IV. The projected 100- and 180-day treatment-related mortality rate for the whole group of patients is 9%.

The post-transplant outcomes at our centre, are in agreement with those reported by experienced centres in Western countries and by international registries (EBMT, CIBMTR).[1,4] It is well-documented that patients in remission or with chemo-sensitive disease have significantly better outcomes as compared with those with refractory or active disease at the time of transplant.[1,4–6] Our patients who have been transplanted in an early disease phase and especially in remission, achieved prolonged OS and PFS rates. However, 3 out of 9 (30%) of allografted patients underwent transplant late, in advanced disease phases, due to 2 major factors. The first was a delay in referral from the primary hospital that initially

Table 1 Patients' and Transplant Procedure Characteristics

	Total HSCT	Autologous HSCT	Allogeneic HSCT
Number	48	39	9
Age (med, range)	45,5 (18–67)	46 (18–67)	45(18–58)
Males	27	23	4
Females	21	16	5
Disease			
Multiple Myeloma	24	24	0
Hodgkin Lymphoma	8	8	0
Non-Hodgkin lymphoma	7	6	1
Gestational Tumor	1	1	0
Acute Myeloid leukaemia	4	0	4
Acute Lymphoblastic leukaemia	2	0	2
Myelodysplastic syndrome	1	0	1
B-Thalassemia Major	1	0	1
Conditioning regimens			
Myeloablative	43	38	5
Reduce intensity	5	1	4
Mobilization			
Chemotherapy + GCSF	17	17	0
Chemotherapy + GSCF+ Plerixafor	4	4	0
GCSF alone	21	12	9
GCSF + Plerixafor	6	6	0
Graft (Peripheral stem cell)			
CD34+ x 10^6 /kg (med, range)	4,6 (2,2–21,4)	4,3 (2,2–21,4)	6 (4,4–8,2)
Donors for alloHSCT			
Full matched siblings			6
Haploidentical			3
Engraftment day (med, range)	12 (9–33)	11 (9–17)	19 (13–33)
Days for full chimerism (for alloHSCT)			30(20–103)
GvHD prophylaxis (for allogeneic HSCT)			
CSP + sort term MTX			4
PTCy + CSP+ MMF			5
Median Follow-up (months)	9,6 (1,2–24)	9,8(1,2–24)	6 (1,9–9,8)
Acute GvHD Grade ≥II	3		3
Chronic GvHD (on 5 evaluable pts)	2		2
Alive Patients	44	38	6
Progressed/relapsed patients	8	7	1
Treatment-related mortality	4	1	3
Alive & non progressed patients	36	31	5

Abbreviations: HSCT, Haematopoietic stem cell transplantation; med, median; GCSF, granulocyte colony stimulating factor; GvHD, graft vs host disease; CSP, cyclosporin; MTX, methotrexate; PTCy, post-transplant cyclophosphamide; MMF, Mycophenolate Mofetil.

managed the patient. This delayed referral was usually related to a prolonged and largely ineffective exposure to conventional chemotherapies, which may contribute to increased disease refractoriness and organ toxicity, thus impairing the beneficial outcome of transplant. The second important factor for not performing HSCT in time was either a delay of acceptance or even multiple rejections from insurance companies, since HSCT is considered a relatively expensive treatment and is covered only by the highest insurance levels in the UAE. It is well known that the higher the insurance level, the better the treatment coverage.[7] However, now that HSCT has become the standard of care for many malignant and non-malignant haematological diseases, it can no longer be considered an "extraordinary" treatment offered only to a minority of patients.

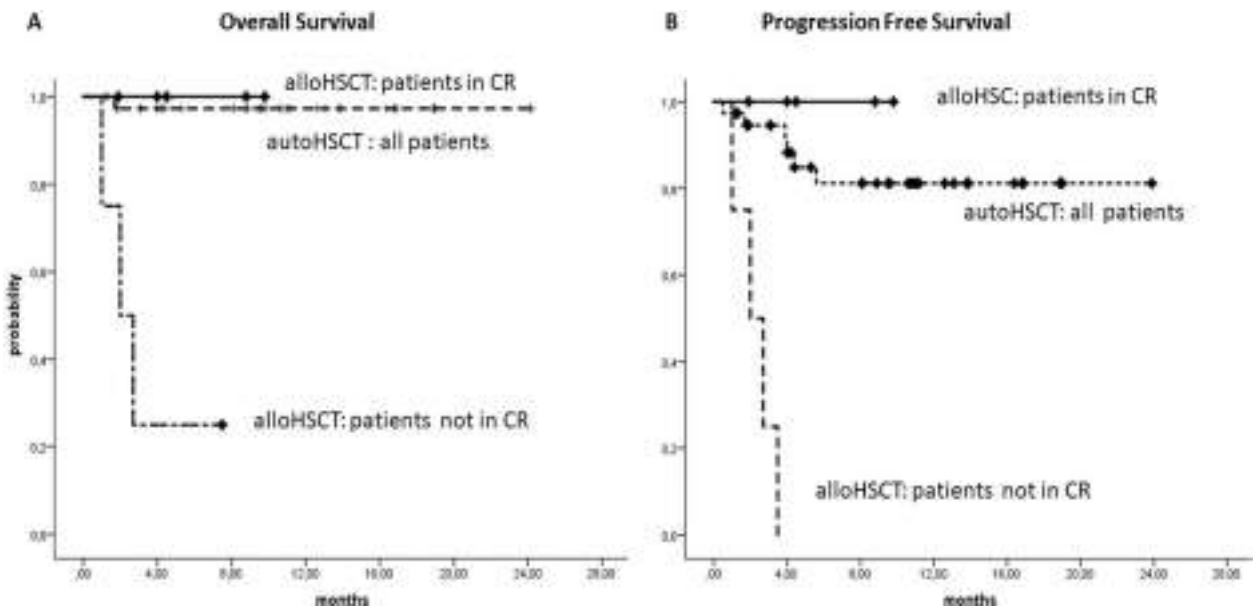

Figure 1 Survival rates for allografted and autografted patients. (**A**) Overall survival rates. (**B**) Progression-free survival rates.

In conclusion, our short-term results confirm that local implementation of HSCT, along with proper post-transplant monitoring in the private sector in the UAE, is a feasible, safe, and effective treatment approach for selected patients with haematological malignancies who have either a full-matched or a haploidentical donor. Better communication with other haemato-oncology centres in the UAE will result in the timely referral of candidate patients to the transplant centre, thus further improving the transplant outcomes. Additional efforts by health authorities and insurance companies will help to update and expand the list of the offered treatment approaches through insurance, so eventually more patients can benefit from HSCT, which is currently worldwide approved as one of the most effective treatment approaches for certain haematological malignant and non-malignant diseases.

Data Sharing Statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Ethics Approval

The Ethics Committee of Burjeel Medical City has approved the study (as it is already stated in the manuscript).

Consent to Participate & Publication

All patients/participants in this retrospective analysis have signed informed consent for using the clinical data for scientific purposes and for publication.

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. They took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; and have agreed for submission to the *Journal of Blood Medicine* and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing financial interests.

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Use and Effectiveness of Carboximaltose Iron in Preoperative Anemia Treatment: A Multicenter and Retrospective Study

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Open Access Full Text Article ORIGINAL RESEARCH

Use and Effectiveness of Carboximaltose Iron in Preoperative Anemia Treatment: A Multicenter and Retrospective Study

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Aim: Anemia, primarily due to iron deficiency, is a key risk factor in both elective and emergency surgeries. Immediate preoperative treatment with ferric carboxymaltose (FCM) in anemic patients can reduce the need for transfusions and the length of hospital stay, thereby optimizing surgical outcomes. The objective of this study was to assess the effectiveness and describe the use of administering intravenous FCM prior to elective scheduled surgery for patients diagnosed with anemia.

Methods: Multicenter, retrospective cohort study that encompassed patients aged 18 years and older who underwent surgery between January 2017 and December 2018. Demographic variables, dose scheme, baseline and perioperative haemoglobin (Hb), transfusion requirements, and admission days were collected. The primary endpoints were the response rate and effectiveness of FCM, defined as the proportion of patients with Hb preoperative levels of ≥13 g/dL. A patient response was deemed to occur when Hb level increased by 1 g/dL or more. The secondary endpoints were the appropriateness of FCM dose, transfusion requirement rate, and length of hospital stay.

Results: 446 patients (55.2% women, median age 69 IQR:52–78 years) were included. The median total dose of FCM administered was 1000 mg over a span of 5 day (IQR: 0–16) days before surgery. 62.8% of patients received lower doses, 24.9% had an INCREASE of Hb ≥ 1 g/dL, 11.6% had Hb ≥ 13 g/dL and 21.3% required blood transfusions, with a mean of 0.73 units transfused. The length of the hospital stay was 12 days (IQR:6–23).

Conclusion: Low percentage of patients achieved a hemoglobin level of 13 g/dL or experienced an increase in hemoglobin of 1 g/dL or more following the administration of FCM, indicating the low effectiveness of FCM in treating perioperative anaemia in our surgical patients. There is underdosing of FCM and insufficient time between FCM administration and surgery in most patients. Both transfused and non-transfused patients show similar Hb increases, while those receiving a standard 1000 mg dose of FCM experience shorter hospital stays compared to those receiving 500 mg, and patients with more transfusions have longer hospital stays.

Keywords: iron intravenous administration, ferric carboxymaltose, iron deficiency anemia

Introduction

Anaemia, as defined by the World Health Organization (WHO), is characterized by a circulating hemoglobin (Hb) concentration below 130 g/L in men and 120 g/L in women. The WHO defines anaemia in children under 5 years of age and pregnant women as a haemoglobin concentration <110 g/L at sea level and anaemia in non-pregnant women as a haemoglobin concentration <120 g/L. According to the WHO, iron deficiency anemia (IDA) is defined as ferritin levels below 15 micrograms/L, with hemoglobin levels under 12 g/dL for nonpregnant females aged 12 and older and under 13 g/dL for males aged 15 and older.[1] Preoperative anaemia can contribute to complications during and after surgery. In several large studies of non-cardiac surgical patients, the prevalence of preoperative anaemia ranged from 10–70% depending on the definition of anaemia and type of intervention. This was found to be higher in oncological and

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gynaecological surgery.[1–4] Preoperative anemia stands as an autonomous risk factor for perioperative blood transfusion, morbidity, and mortality.[4,5] In surgical patients, the administration of red blood cell transfusions (RBCT) may be associated with an increased risk of infection, circulatory overload, and thromboembolic events. Additionally, it can lead to prolonged hospitalization, impaired quality of life, and should therefore be minimized or avoided whenever possible.[1,6]

Since preoperative haemoglobin concentration is a major predictor of perioperative transfusion, haemoglobin optimisation is a key aspect of patient blood management. The toxicity concerns from EPO erythropoietin (EPO) would reinforce the role of IV iron. Indeed, the majority of guidelines from professional associations and international consensus documents endorse the use of intravenous iron in the management of perioperative anemia.[5,7–15] Prior research has indicated that administering intravenous ferric carboxymaltose (FCM) treatment a minimum of 1 week prior to surgery elevates hemoglobin levels and, thereby, is expected to decrease the necessity for RBCT during the perioperative phase.[4,6,10,16–18] Various intravenous iron formulations are commercially available, and research has demonstrated the safety and efficacy of all these formulations, depending on the dose, for correcting anaemia. Intravenous FCM is the predominant treatment for iron deficiency anemia in Spain. It enables the administration of high iron doses (up to 1000 mg) through short infusions, with a low risk of severe anaphylactic reactions (incidence approximately 1 in 250,000 administrations). Iron carboxymaltose is ideal for treating perioperative anemia because it quickly replenishes iron stores, crucial for surgical patients needing rapid recovery. Its intravenous administration ensures effective absorption, bypassing gastrointestinal issues often associated with oral iron. This reduces the risk of delays or complications due to anemia. Additionally, fewer doses improve patient compliance and streamline pre-surgical preparation. Its safety profile and efficiency make it suitable for managing anemia in the perioperative setting.[5,19,20]

Iron deficiency anemia is a surprisingly common condition in various surgical and other patient populations. Even among patients whose anemia is attributed to other causes (eg, chronic kidney disease or inflammation), there may be some degree of iron deficiency. People with iron deficiency should be treated with iron rather than transfusions, unless the anemia is extremely severe and there is a risk of organ ischemia, as discussed separately. If iron is administered, sufficient time should be allowed for effective treatment of anemia before surgery (usually two to four weeks for partial correction and six to eight weeks for complete correction). In people with unexplained iron deficiency, determining the underlying cause is an essential part of treatment.

Another study concludes that intravenous iron is an effective intervention to improve Hb concentration in patients with iron deficiency anemia, despite the majority of patients not receiving the full dose based on their baseline Hb level and weight. Increasing the interval time between infusion and surgery was associated with a greater increase in Hb, with only a minimal increase observed if given less than 2 weeks prior to surgery.[21]

Oral iron replacement can be started in a patient with iron deficiency if at least four to six weeks are available before the planned surgery. Intravenous (IV) iron is an option if there are fewer than four to six weeks until the scheduled surgery and for patients who cannot tolerate oral iron or do not respond (eg, due to malabsorption). In this context, intravenous iron can replenish the body's iron stores more quickly and effectively than oral iron treatment; however, time is still needed for the iron to incorporate into developing red blood cells and for the hemoglobin level to rise, and Although it has been observed that in certain cases FCM administration was associated with a reduced need for perioperative transfusion and can safely stabilize hematological parameters,[22,23] the available trials do not have sufficient statistical power to determine if transfusion rates are indeed lower.

Given this context, the present study was formulated to evaluate the effectiveness of administering preoperative FCM in patients undergoing surgery for deficiency anaemia. The secondary endpoints included appropriateness of the doses, transfusion requirement rate, and length of hospital stay.

Materials and Methods

Ethical approval for this study was obtained from the Ethics Committee of CEIm 2019.10. EO provided by the Ethical Committee of Guadalajara University Hospital, Guadalajara, Spain, chaired by Prof. Juan Ramon Urbina Torija, on February 12, 2019 (Investigation Protocol Number: PR-FE-01). This committee reviewed the study upon which this manuscript is based and granted approval to waive the requirement for informed consent based on the principles outlined in the Declaration of Helsinki. All patient information is confidential and protected under applicable privacy laws.

Unauthorized access, use, or disclosure of patient data is strictly prohibited and may result in disciplinary action and legal consequences. We are committed to ensuring the privacy and security of all patient records.

This multicentre cohort retrospective study was conducted at three Spanish hospitals: Guadalajara University Hospital (UGH), Virgen del Rocío University Hospital (UVRH) and Vall d'Hebron Hospital (UVEH). Consecutive non-probability sampling was used. The institutional review board granted approval for this study.

The study population comprised individuals aged 18 years and older who underwent elective surgery and were treated with FCM between January 2017 and December 2018 (24 months). There were no restrictions on the surgical procedures. The study excluded patients who underwent emergency surgery and individuals with concurrent illnesses associated with anemia, such as hematological and oncological conditions or renal failure, and those lacking perioperative hemoglobin data (preoperative Hb and Hb in hospital stay). Emergency surgery was defined as a medical emergency necessitating immediate surgical intervention, where postponement was not feasible for successful resolution.

The primary endpoints were the response rate and effectiveness of FCM, defined as the percentage of patients with preoperative hemoglobin levels of 13 g/dL or higher (regardless of gender). We defined preoperative hemoglobin as the last hemoglobin measurement before the index operation. A patient's response was deemed to occur when the hemoglobin (Hb) level increased by at least 1 g/dL or if the Hb level reaches ≥13 g/dL. In this study, a hemoglobin level of 13 g/dL was considered appropriate for both sexes, supported by numerous studies. It may no longer be justifiable to use a lower hemoglobin threshold to define anemia in women, as this increases the risk of adverse outcomes and the need for costly transfusions. The definition of anemia should be standardized across sexes, especially in the perioperative setting, with a hemoglobin level below 130 g/L requiring intervention for both men and women.[2,24–28]

The secondary endpoints were the appropriateness of FCM doses, transfusion requirement rate, and length of hospital stay.

Demographic variables (sex and age), weight, surgical procedure, bleeding risk, dose scheme, baseline and perioperative hemoglobin (Hb) levels, preoperative ferritin levels, transfusion requirements, and days of hospital admission were collected from the electronic health records. We defined baseline hemoglobin as the hemoglobin value on the day of the preoperative anesthesia consultation when FCM was administrated. Analytical variables were collected before FCM administration in the perioperative period and at discharge. Iron deficiency was delineated based on the criteria set forth by the World Health Organization (WHO).[29] The risk of bleeding was classified as high, moderate, or low based on the type of intervention. (according to the Consensus Document of the Spanish Society of Cardiology, 2018: "Perioperative management and periprocedural antithrombotic treatment").[30]

The appropriateness of FCM doses was evaluated according to the Ganzoni Formula [Iron dose (mg)= [target haemoglobin (Hb) (g/dL) – actual haemoglobin (Hb) (g/dL)] x weight (Kg) x 2.4 + iron deposits (500 mg in adults); target Hb: 13 g/dL], and FCM Summary of Product Characteristics (SPC).[31] For the administration of intravenous iron in surgeries planned in less than a month, the FCM dose must be calculated using the Ganzoni Formula (FG). Patients without weight data were estimated by considering an average weight of 70 kg for men and 65 kg for women. The time between the administration of FCM and surgery is defined as the number of days between the day the dose of ferric carboxymaltose is administered (along with the corresponding lab tests) and the day the patient undergoes surgery (also with the corresponding lab tests).

It is recommended to apply "restrictive" transfusion criteria for red blood cell concentrates (RBCs) in most hospitalized patients (medical, surgical, or critical) without active bleeding and who are hemodynamically stable (including those with sepsis, upper gastrointestinal bleeding, or postpartum anemia) if they present symptoms or an Hb level <7.0 g/dL. For cardiac surgery patients, restrictive transfusion criteria are recommended at an Hb ≤7.5 g/dL. For patients with a history of cardiovascular disease undergoing orthopedic surgery or hip fracture repair, restrictive transfusion criteria are recommended at an Hb <8.0 g/dL.[32–44]

Statistical Analysis

Baseline characteristics were described using the median with interquartile range (IQR) for continuous data and percentages for categorical data. Mean and standard deviation were utilized for normally distributed continuous data. Qualitative variables were compared using the $\chi 2$ test, with Fisher's exact test applied if expected counts were below five. For quantitative variables, comparisons were conducted using either the *t*-test or ANOVA for normally distributed data,

while the Mann–Whitney *U*-test or Kruskal–Wallis test was used for non-normally distributed data. Logistic regression analysis was employed to evaluate the association between FCM administration and increased Hb levels, reported as odds ratios (OR) with 95% confidence intervals (CI). All statistical tests were two-tailed, with significance set at a p-value <0.05.

Statistical analysis was conducted utilizing the SPSS statistical software package v.15 for Windows and STATA v.16 for Mac.

Results

Baseline and Clinical Characteristics

A total of 446 of 506 patients treated with FCM were included in this study. Sixty patients did not have any perioperative Hb data and were excluded. Two hundred forty-six (55.2%) patients were female, and the median age was 69 (IQR 52–78) years. Baseline characteristics, surgical procedures, and proportion of patients with preoperative bleeding are shown in Table 1.

Table 1 Baseline Characteristics, Surgical Procedure and Bleeding Risk

	Total	UGH	UVRH	UVEH	
Demographic characteristics					
Sex	446	103	71	272	
Females (%, n)	55.2% (246)	54.4% (56)	67.6% (48)	52.2% (142)	p=0.066
Age (median; IQR)	69 (52–78)	72 (59–82)	58 (46–71)	70 (53–78.8)	p=0.052
Prevalence of anaemia					
Total	84.3% (376)	93.2% (96)	98.6% (70)	77.2% (210)	
Women (Hb<12 g/dL)	76.8% (189)	89.3% (50)	97.9% (47)	64.8% (92)	p=0.466
Men (Hb<13 g/dL)	93.5% (187)	97.9% (46)	100% (23)	90.8% (118)	p= 0.427
Prevalence of iron deficiency anaemia (IDA): Only 67.8% (255) patients had ferritin data.					
Total (ferritin<15 µgrams/L)	2.1% (8)	4.2% (4)	No data	1.9% (4)	
Surgical Procedure (SP)					
Gastrointestinal	39.7% (177)	62.1% (64)	46.5% (33)	29.4% (80)	p<0.001
Orthopaedic	17% (76)	4.9% (5)	9.9% (7)	23.5% (64)	
Cardiovascular	15.5% (69)	4.9% (5)	16.9% (12)	19.1% (52)	
Gynaecological	6.5% (29)	16.5% (17)	8.5% (6)	2.2% (6)	
Urologic	2% (9)	2.9% (3)	0.0% (0)	2.2% (6)	
Ophthalmic	0.9% (4)	3.9% (4)	0.0% (0)	0.0% (0)	
Other	18.4% (82)	4.9% (5)	18.3% (13)	23.5% (64)	
Bleeding risk					
Total (excluded=6)	98.7% (440)	99.0% (102)	97.2% (69)	98.9% (269)	
Low	9.5% (42)	17.6% (18)	10.1% (7)	6.3% (17)	p<0.001
Moderate	52.5% (231)	61.8% (63)	43.5% (30)	51.3% (138)	
High	38.0% (167)	20.6% (21)	46.4% (32)	42.4% (114)	

Abbreviations: IQR, interquartile range; UGH, Guadalajara University Hospital (Guadalajara); UVRH, Virgen del Rocío University Hospital (Seville); UVEH, Vall d'Hebron Hospital (Barcelone).

The overall prevalence of anaemia was 84.3% (n=376): 93.5% in males (Hb<13 g/dL) and 76.8% in females (Hb<12 g/dL). The UVEH group had a significantly lower number of patients with anaemia (77.2%). Prevalence of iron deficiency anaemia (IDA) was 2.1%, with ferritin data available for only 67.8% (255) of patients. Ferritin data was unavailable for the UVRH group.

The most common procedures performed in the total population were gastrointestinal surgery (39.7%), orthopedic surgery (17%), cardiovascular surgery (15.5%), and excluding gynecological surgery (6.5%). As presented in Table 1, patients treated with UGH and UVEH more commonly presented a moderate bleeding risk, whereas those treated with UVRH presented a high bleeding risk.

Primary Outcomes

At the time of data collection, the median baseline Hb (bHb) were 10.5 g/dL (IQR 9.6 11.7). The evaluation of haemoglobin levels is presented in Table 2.

Regarding the increase in Hb achieved, 11.6% of patients had preoperative Hb ≥13 g/dl, and 52.5% (21) had high-risk bleeding. Overall, the response rate, defined as bHb ≥1 g/dL, was 24.9% (excluding patients with preoperative Hb ≥13 g/dl), with the highest proportion in the UVRH group (50.7%). The mean increase in Hb levels compared to baseline was 0.28 g/dL (SD 0.09).

FCM was administered 5 (IQR, 0–16) days before surgery, and 15.3% (68) of patients had more than four weeks between FCM administration and surgery, allowing sufficient time for erythropoiesis and iron use (FCM SPC). There was large variability between hospitals. UVRH showed longer periods between administration and surgery (24 days; IQR 9.75 43.5).

Secondary Outcomes

The median FCM dose administered was 1000 mg (IQR 500–1000). There were no significant differences among the three hospitals (p>0.05). A total of 280 (62.8%) patients received lower dosages according to the FCM SPC recommendations. According to the Ganzoni Formula, 20.9% (93) of patients received higher doses than needed: between 500–1000 mg in 17.6% (78) of patients and higher than 1000 mg in 3.4% (15) of patients.

The doses received by FCM are shown in Table 3.

This table titled “Ferric carboxymaltose dosage according to Summary of Product Characteristics” presents data on the actual doses of FCM administered to patients compared with the doses recommended in the technical guidelines. The data is stratified by patients’ weight (35–70 kg and >70 kg) and hemoglobin (Hb) levels (<10 g/dL, 10 to <14 g/dL, and ≥14 g/dL).

For patients with Hb < 10 g/dL and weighing 35–70 kg, the recommended FCM dose was 1500 mg, but only 8.5% of these patients received this dose, with the majority receiving either 500 mg (38.8%) or 1000 mg (48.8%). In those weighing more than 70 kg, the recommended dose was 2000 mg, yet 72.4% received only 1000 mg. Among patients with Hb 10 to <14 g/dL, for those in the 35–70 kg group, the recommended dose was 1000 mg, but nearly half (49.8%) received 500 mg, and 45.7% received the full 1000 mg dose. In the >70 kg group, the recommended dose was 1500 mg, with 68.8% receiving 1000 mg and 29.2% receiving 1500 mg. For patients with Hb ≥ 14 g/dL in the 35–70 kg group, the recommended dose was 500 mg, which was received by the majority (52.6%), while some received higher doses of 1000 mg (36.8%) or 1500 mg (10.5%), and no patients in the >70 kg category with Hb ≥ 14 g/dL were included in this dataset.

In the analysis of transfusion requirements (Table 2), 95 patients (21.3%) required transfusion after FCM administration, and the mean number of RBC units transfused was 0.73, with less in the UVEH group (13.2% and 0.26 units). We analysed a subgroup of patients in need of transfusion according to an increase in bHb ≥1 g/dL, dosage received, and the risk of bleeding. Overall, 27.4% of patients with an increase in Hb ≥1 g/dL were transfused compared to 72.6% of patients with an Hb variation <1 g/dL (p=0.23), and 27.4% of patients who received 500 mg FCM required transfusion compared to 57.9% of patients who received 1000 mg FCM (p=0.003). Regarding the risk of bleeding, 37.9% of patients with a high bleeding risk needed transfusion compared with 61.0% of patients with a moderate bleeding risk requiring transfusion compared with 1.0% of patients with a low bleeding risk requiring transfusion (p<0.05).

The median duration of hospitalization (Table 2), calculated from the day of surgery to discharge, was recorded as 12 days (IQR: 6–23 days). A subgroup analysis showed a median of 15 days (IQR 8–31) in patients who received

Table 2 Evolution of Haemoglobin, Transfusion Requirement and Hospital Stay

	Total	UGH	UVRH	UVEH	
Evolution of haemoglobin					
bHb Median (IQR) (g/dL)	10.5 (9.6–11.8)	10.7 (9.6–11.7)	9.9 (9–10.5)	10.8 (9.8–12)	p<0.001
Hb perioperative median (IQR) (g/dL)	10.9 (9.8–12.1)	11.3 (10.1–12.3)	11 (9.8–12)	10.7 (9.7–12)	p=0.250
Hb preoperative ≥ 13 g/dL (%, n)	11.6% (52)	19.2% (10)	13.5% (7)	67.3% (35)	p=0.622
Increase bHb ≥1 g (dL) (%, n)	24.9% (111)	34.0% (35)	50.7% (36)	14.7% (40)	p<0.001
Mean increase Hb (SD)	0.28 (SD 0.09)	0.61 (SD 0.15)	1.09 (SD 0.23)	−0.06 (SD 0.11)	p=0.028
Time between FCM and surgery (median; IQR) (days)	5 (0–16)	10 (5–21)	24 (9.75–43.5)	1 (0–8)	p<0.001
Transfusion					
Need for transfusion (patients) (%, n) Mean units transfused (SD) 500 mg FCM 1000 mg FCM 1500 mg FCM 2000 mg FCM	21.3% (95) 0.73 (1.9) 27.4% (26) 57.9% (55) 8.4% (8) 6.3% (6) p=0.003 (vs Need for transfusion patients)	33.0% (34) 1.51 (2.8) 5.8% (2) 91.2% (31) – 3.0% (1)	35.2% (25) 1.51 (2.7) 0.0% (0) 56.0% (14) 24.0% (6) 20% (5)	13.2% (36) 0.26 (0.7) 66.7% (24) 27.8% (10) 5.5% (2) 0.0% (0)	p<0.001 p<0.001 p<0.001
Low risk bleeding Moderate risk bleeding High risk bleeding	1.0% (1) 61.0% (58) 37.9% (36) p<0.05	0.0% (0) 76.5% (26) 23.5% (8)	4.0% (1) 44.0% (11) 52.0% (13)	0.0% (0) 58.3% (21) 41.7% (15)	p=0.070
bHb≥1 mg/dL bHb<1 mg/dL	27.4% (26) 72.6% (69) p=0.23 (vs Need for transfusion patients)	72.6% (11) 27.4% (23)	28.0% (7) 72.0% (18)	22.2% (8) 77.8% (28)	p=0.479
Hospital stay					
Median Time (days, IQR)	12 (6–23)	8 (5–14)	5 (3–11)	16 (8–31.8)	p<0.001
Patients with transfusion (days, IQR) Patients without transfusion (days, IQR)	15 (8–31) 10 (6–20)	12.5 (8–19.5) 6 (4.7–9.25)	10 (6.50–20.00) 4 (2–7)	28.50 (14.25–53.00) 15 (8–27.75)	–
500 mg FCM (days, IQR) 1000 mg FCM (days, IQR) 1500 mg FCM (days, IQR)	16 (8–32) 9 (5–19) 8 (4–18)	9.50 (4.50–43.75) 8 (5–14) –	– 5 (2.25–9) 5 (4–10)	16 (8–32) 16 (8–31) 25.5 (12–54.75)	–
Low risk bleeding (days, IQR) Moderate risk bleeding (days, IQR) High risk bleeding (days, IQR)	13 (6.75–34.25) 11 (6–21) 12 (6–25)	8.50 (5.75–25.00) 8 (5–13) 8 (4–18)	5 (4–7) 6.50 (3–13.50) 4.50 (2–10.75)	21 (12.50–38.00) 14.50 (8–30) 16 (8–32.25)	–

Abbreviations: FCM, ferric carboxymaltose; IQR, interquartile range; UGH, Guadalajara University Hospital; UVRH, Virgen del Rocío University Hospital; UVEH, Vall d'Hebron Hospital; Hb, haemoglobin; bHb, Hb before FCM administration.

a transfusion versus 10 days (IQR 6–20) in those who did not (p<0.05). The length of hospital stay was similar according to the risk of bleeding (13 days with low risk, 11 days with moderate risk, and 12 days with high risk). The median hospitalisation time was 16 days among patients who received 500 mg FCM and 9 days among patients who received 1000 mg FCM (p<0.05).

Table 3 Ferric Carboxymaltose Dosage According to Summary of Product Characteristics

Weight (*)	35–70 Kg Weight		>70 Kg Weight	
Hb (g/dL) (n)	**FCM SPC Dose**	**Received Dose (n, % Patients)**	**FCM Dose (SPC)**	**Received Dose (n, % Patients)**
<10	1500 mg	500 mg (50, 38.8%) 1000 mg (63, 48.8%) **1500 mg (11, 8.5%)** 2000 mg (4 3.1%) 3000 mg (1, 0.8%)	2000 mg	1000 mg (21, 72.4%) 1500 mg (3, 10.3%) **2000 mg (5, 17.2%)**
10 a <14	1000 mg	500 mg (110, 49.8%) **1000 mg (101, 45.7%)** 1500 mg (3, 1.4%) 2000 mg (7, 3.2%)	1500 mg	1000 mg (33, 68.8%) **1500 mg (14, 29.2%)** 2000 mg (1, 2.1%)
≥14	500 mg	**500 mg (10, 52.6%)** 1000 mg (7, 36.8%) 1500 mg (2, 10.5%)	500 mg	No patients

Notes: (*) No patients with weight <35 Kg. Bold text included is recommended dose.
Abbreviations: FCM, ferric carboxymaltose; SPC, Summary of Product Characteristics; Hb, haemoglobin.

Discussion

We found that anaemia, as defined by the WHO Health Organization criteria, was highly prevalent in patients preoperatively. From this perspective, we aimed to determine whether preoperative FCM administered before surgery would correct the underlying iron deficits. We also aimed to understand the use of FCM and its impact of FCM administration in clinical outcomes.

In the effectiveness analysis, almost 25% of the patients had an increase in Hb levels in the preoperative period compared with bHb ≥1 g/dL after the administration of FCM. In comparison to standard care, intravenous iron resulted in a notable increase in hemoglobin levels by 0.8 g/dL, contrasting with the 0.1 g/dL improvement observed with conventional treatment, as demonstrated by a randomized controlled trial encompassing 72 patients undergoing major abdominal surgery (p=0.01) upon admission.[6] One systematic review discovered that among a subgroup of anemic patients undergoing colorectal surgery, there was a slightly greater increase in hemoglobin at the conclusion of preoperative treatment with intravenous iron compared to placebo. However, this difference was not statistically significant nor clinically relevant.[13]

It is important to note that 11.7% of the patients were treated with FCM and had no anaemia (preoperative Hb level ≥13 g/dl). They were excluded from the effectiveness analysis; however, more than half of these patients had a high risk of bleeding.

The prevalence of iron deficiency anaemia (IDA) observed in this study was relatively low at 2.1%, which could be interpreted in several ways. First, it may suggest effective iron management within the patient population. However, the limited availability of ferritin data (only 67.8% of patients) raises concerns about potential underdiagnosis. Ferritin is a key marker for diagnosing IDA, and the absence of this data in over 30% of patients suggests that the true prevalence could be higher than reported.

Based on our findings, approximately 90% of the patients treated with FCM had a high or moderate risk of bleeding. The median dose received was 1000 mg, and almost a quarter of the patients received higher doses than needed, according to the Ganzoni Formula, and approximately 70% of the patients received an inadequate dosage according to the FMC SPC recommendations. In the preoperative period following FCM administration, a quarter of the patients experienced a rise in Hb level of at least 1 g/dL. Almost a quarter of the patients required transfusion.

While ideally, the intravenous iron dosage should be calculated based on the total body iron deficit using the Ganzoni formula, in clinical practice, this calculation is of limited relevance due to the maximum allowable iron dose being restricted to 1000 mg. In our study, almost a quarter of the patients received a dose higher than the ideal dose according

to the Ganzoni formula. UVEH did not collect patient weight data, and considered an average weight of 70 kg for men and 65 kg for women to calculate the FCM dose using the Ganzoni Formula. This may have influenced the mean dose used by patients in this study.

In relation to Table 3, which analyzes the discrepancies between recommended and administered doses of Ferric Carboxymaltose (FCM) in patients based on their hemoglobin levels and body weight. The data reveal a significant discrepancy between the recommended doses of FCM according to technical guidelines and the actual doses administered to patients. For patients with Hb < 10 g/dL, the recommended doses were often not met, particularly in the higher weight category where a substantial number of patients received only half of the recommended dose. This trend is consistent across all Hb levels, where lower than recommended doses were frequently administered. The variation in dosing, particularly the administration of doses lower than those recommended, could be due to a variety of factors including clinical judgment, concerns about side effects, or limitations in resource availability. However, this discrepancy raises concerns about the potential for under-treatment, especially in patients with severe anemia, which could impact their recovery and overall outcomes.

The international statement on the perioperative management of anaemia[14] suggests oral iron administration when there is a suitable interval before surgery, typically ranging from 6–8 weeks, and when there are no contraindications present. In our study, we did not record whether the patients had taken oral iron prior to intravenous iron administration, but the average time between FCM administration and surgery was not sufficient to obtain an adequate Hb increase. The average time spent in the three hospitals was 5 days. Prior research has demonstrated that the administration of intravenous iron at least one week prior to surgery elevates hemoglobin levels, thereby potentially decreasing the requirement for RBCT units during the perioperative period.[10,17,45]

In relation to RBCT, both the percentage of patients and the quantity of units transfused were notably higher compared to those reported in other studies. In Calleja et al[16] out of the subgroup of patients with colon cancer who received preoperative FCM, only 9.9% were transfused and 0.2 units transfused. In Bisbe et al's controlled trial, Just 7% of patients in the FCM group necessitated RBCT transfusion.[45] In our study, patients who received transfusions spent more days in the hospital compared to those that did not, with statistically significant result (p=0.003). Likewise, increased length of hospital stay in patients who received transfusions has been reported in other studies.[7,46] Despite this, the Hb increase in transfused patients was similar to that in non-transfused patients.

This study had several limitations that warrant acknowledgement. First, it had a retrospective and non-comparative design with a surgical population that did not receive FCM. It is not possible to determine with certainty whether the improvement in perioperative outcomes was due to FCM. Additionally, nearly 20% of patients lacked hemoglobin data during the perioperative period. This may have led to potential bias in the interpretation of the results.

Our study, however, has several strengths, particularly the large sample size of patients considered for the analysis, the participation of three large Spanish hospitals, and the representation of "real-life" clinical practice.

Conclusions

In conclusion, there was a low proportion of patients achieving a hemoglobin level of 13 g/dL or experiencing an increase in hemoglobin of 1 g/dL or more following the administration of FCM, which indicates the low effectiveness of FCM in treating preoperative anaemia in our surgical patients. Future randomised controlled trials focused on evaluating the effectiveness of FCM treatment for preoperative anaemia may support our findings.

Patients are regularly underdosed with FCM and the time between FCM administration and the date of surgery is insufficient for most patients. It is necessary to develop a protocol for the management of FCM to ensure adequate prescription and include any iron study as a factor in determining who to administer IV iron. The findings suggest that the administration of FCM in clinical practice often falls short of the recommended guidelines, particularly in patients with more severe anemia. This under-dosing could have significant implications for patient outcomes, indicating a need for further investigation into the reasons for these discrepancies and potentially a reassessment of clinical practices to ensure that patients receive the appropriate dosage of FCM as per the guidelines.

The lack of comprehensive ferritin data may have led to an underestimation of IDA prevalence, as patients without this data might still have had undetected iron deficiency. Therefore, the reported 2.1% prevalence should be considered with caution, and future studies should aim for more complete data collection to ensure accurate prevalence estimates.

Additionally, this finding highlights the importance of improving data completeness in clinical practice to enhance the reliability of study outcomes. Information about inflammation and other iron data status would be useful to gauge response to iron therapy. Lack of this information is a limitation.

The observation that the increase in Hb in transfused patients was similar to that in non-transfused patients reflects that the effectiveness in terms of Hb level increase does not differ significantly between the two groups.

Patients who receive a higher number of transfusions experience longer hospital stays. Patients who receive a standard dose of 1000 mg of FCM have shorter hospital stays compared to those who receive 500 mg.

Disclosure

The authors report no conflicts of interest in this work.

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Blood Donation: Fears and Myths in Healthcare Workers of the Future

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Blood Donation: Fears and Myths in Healthcare Workers of the Future

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Objective: To determine the fears and myths related to blood donation in future health care workers.

Study Design: Cross-sectional study.

Place and Duration of Study: This study was carried out from October to December 2022 at the National University of Medical Sciences (NUMS), Rawalpindi, Pakistan. Donors were selected according to the, WHO recommended, Safe Blood Transfusion Program of Pakistan criteria.

Results: In total, 411 participants were included in the study. The individuals were 21–24 years of age, with a mean age of 21 years. In our study, females dominated (232/411); the remaining 179 were males. Out of the total 411, 145 participants had previously donated blood while the other 266 had never donated blood. Our study analyzed both of these groups. The most common symptoms experienced by blood donors were dizziness, post-donation weakness, and bodily aches and pains. Most non-donors feared problems related to their general health (42.3%) and developing infections (12.7%). *P*-value was 0.002, which reveals a significant association between fears and intention to donate blood.

Conclusion: These results suggest that fears and concerns related to blood donation play a leading role in forecasting donors' attitudes and intentions. Motivation leads to inspiration and potential donors can be motivated by addressing their fear.

Keywords: blood donation, fears, myths, healthcare workers

Introduction

According to the World Health Organization, the overall rate of blood donation is 31.5 donations in high-income countries, 16.4 donations in upper-middle-income countries, 6.6 donations in lower-middle-income countries, and 5.0 donations in low-income countries.[1] Although, in developed countries, blood management initiatives have successfully managed to decrease the demand for blood products, recent global environmental and biological changes have increased demand for blood and blood products.[2] This was especially the case during the COVID-19 pandemic and Dengue fever outbreaks in our resource-strained country.[3] The demand for blood components like fresh frozen plasma and platelets has increased markedly; thus, encouraging and improving the recruitment and retention of donors remains a high priority.[4] This could be because of increases in the size of the general population, elderly individuals, hematological malignancies, and road traffic accidents.[5]

Young medical students, who are the healthcare workers of the future, should be motivated to donate blood because they are well aware of the misery patients experience.[6] In this study we wanted to identify and document the reasons why some of them do not donate blood. Worldwide, advertising campaigns emphasize the positive aspects of donating while minimizing any possible negative aspects and busting the myths associated with it.[7] To boost recruitment of blood donors, it is important to address the negative aspects directly.[8] In developed countries, donors are unpaid volunteers; in contrast, developing countries have limited supplies so people only donate blood when a family member or friend needs it.[9]

Blood donation is associated with different variables like fear of needle prick, blood-borne diseases, and vasovagal reactions, and issues related to donor recruitment and retention.[10] This study was done to assess medical students' awareness of the need for blood, the donation process, and the impact of their donations on the community. It also explored the attitude of students towards blood donation so as to provide insights into their perceptions and beliefs. Investigating the reasons why

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some students choose not to donate blood is essential. Common barriers could include fear, lack of time, or concerns about health implications.[11] Addressing these reasons can help in developing strategies to overcome these barriers.

Materials and Methods

A cross-sectional study was conducted at the department of Pathology, National University of Medical Sciences, Rawalpindi for a duration of three months, i.e. from October to December 2022. A non-probability consecutive sampling technique was used. Medical and dental undergraduate students attending the National University of Medical Sciences, aged between 21 and 24 years, were included in the study. Students not willing to participate in the study were excluded. Incompletely filled forms were also rejected. The sample size was approximately 411 participants.[12] The study was approved by the ethics review committee of the National University of Medical Sciences. Our study complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all students who took part. Donors were selected according to the WHO recommended Safe Blood Transfusion Program. A detailed history was taken from the students by means of proformas completed by them. The proforma included demographic data such as students' age and gender, history of blood donation, and donation by family members; the time interval between blood donations was also noted. Their responses were stated as yes/no. In our blood collection center, donors are selected according to the WHO criteria that they bleed; components are prepared followed by hemovigilance. In the case of blood donation, a history of headache, dizziness, palpitations, anxiety, vasovagal syncope, post-donation weakness and bodily aches and pains were noted on the proforma. If there was no history of blood donation, then any cultural issues or fears related to needle prick, contacting infections, or problems related to general health were noted. The proforma also included questions related to myths about blood donation, such as fear of weight gain, specific diet plans, or contracting diseases, or any recent vaccinations that might halt the blood-donation process. Statistical analysis was done using SPSS version 22. Descriptive statistics were used for gender distribution; for age, a percentage was calculated. Associations among gender and blood donations were determined using the chi-square test. A chi-square test was also used to evaluate the association of each student's response with their blood donation status. A p-value of < 0.05 was considered to be statistically significant.

Results

A total of 411 students participated in the study. The students were 21 to 24 years of age, with a mean age of 21 years. In our study females dominated (232/411). Out of the total 411 students, 143 had previously donated blood while 268 students had never donated blood. In our study, we analysed both groups. We were interested to know whether the participants who had donated blood experienced any post-donation symptoms and, if so, what was the most common symptom they experienced. Of all participants, 270 had family members who were also donors. (See Tables 1–9 and Figure 1.)

Table 1 Time Interval Between Blood Donations

Sr. No	Time period	N (%)
1	1 year	17 (4.1)
2	2 months	7 (1.7)
3	2 years	8(1.9)
4	3 months	14 (3.4)
5	4 months	6 (1.5)
6	5 months	8 (1.9)
7	6 months	32 (7.8)
8	0	305 (74.2)
9	Once	14 (3.4)

Table 2 Following Blood Donation, Symptoms Experienced by Donors

Sr. No	Symptoms	N (%)
1	Headache	26 (17.1)
2	Dizziness	49 (32.2)
3	Palpitations	10 (6.6)
4	Anxiety	13 (8.6)
5	Vasovagal syncope	5 (3.3)
6	Post-donation weakness, bodily aches and pains	49 (32.2)
Mean±SD	3.45±1.986	

Table 3 Reasons for Not Donating Blood

Sr. No	Reasons	N (%)
1	Cultural issues	5 (1.2)
2	Fear of needle prick	37 (9.0)
3	Fear of contracting infections	52 (12.7)
4	Fear of problems related to general health	174 (42.3)

Table 4 Influencing Factors

Sr. No	Do you think that your decision regarding blood donation can be influenced by the following factors:	N (%)
1	You are on any type of medication (multivitamins/antibiotics)?	53 (12.9)
2	Fear of weight gain?	42 (10.2)
3	If you are on specific diets?	28 (6.8)
4	Any active disease?	155 (37.7)
5	If you have received any type of vaccine recently?	41 (10)

Table 5 Association Between Previous History of Blood Donation and Gender (Significance)

	Previous history of blood donation		P-value
	Yes	No	
Male	98 (54.7%)	81 (45.3%)	0.000
Female	47 (20.3%)	185 (79.7%)	

Discussion

According to our hypotheses, stronger medical fears were associated with lower intention to donate blood; this effect was influenced by attitudes to donation as well as self-confidence (e.g. belief in one's ability to manage fear).[13] In terms of predictors of lower blood donation rates, blood-related fears were among the strongest, despite the fact that fear of needles and venipuncture pain are very noticeable and common concerns during blood donation, these fears strongly contribute to why fewer people donate blood.[14] Seeing blood being drawn from your arm and accumulating in a large

Table 6 Association Between History of Donation in Family Members and Gender (Significance)

	History of donation in family members		P-value
	Yes	No	
Male	105 (59.7%)	71 (40.3%)	0.023
Female	161 (69.7%)	70 (30.3%)	

Table 7 Associations Between Gender, History of Donation in Family Members, and Previous History of Blood Donation

Following blood donation, have you ever experienced the following symtoms?								P-value
		Headache	Dizziness	Palpitations	Anxiety	Vasovagal syncope	Post-donation weakness, bodily aches, and pains	
Gender	Male	17 (20.5%)	22 (26.5%)	6 (7.2%)	8 (9.6%)	0 (0)	30 (36.1%)	0.066
	Female	9 (13.0%)	27 (39.1%)	4 (5.8%)	5 (7.2%)	5 (7.2%)	19 (27.5%)	
History of donation in family members	Yes	16 (15.7%)	34 (33.3%)	5 (4.9%)	8 (7.8%)	3 (2.9%)	36 (35.3%)	0.670
	No	10 (20.4%)	14 (28.6%)	5 (10.2%)	5 (10.2%)	2 (4.1%)	13 (26.5%)	
Previous history of blood donation	Yes	21 (21.4%)	24 (24.5%)	6 (6.1%)	5 (5.1%)	4 (4.1%)	38 (38.8%)	0.006 Significant
	No	5 (9.3%)	25 (46.3%)	4 (7.4%)	8 (14.8%)	1 (1.9%)	11 (20.4%)	

Table 8 Association Between Previous History of Blood Donation & Gender with Reasons for Not Donating Blood (1st Significant & 2nd Not Significant)

		Religious issues	Fear of needle prick	Fear of contracting infections	Fear of general health-related problems	P-value
Previous history of blood donation	Yes	1 (0.7%)	9 (6.2%)	17 (11.7%)	39 (26.9%)	0.000
	No	4 (1.5%)	28 (10.5%)	35 (13.2%)	135 (50.8%)	
Gender	Male	3 (1.7%)	14 (7.8%)	20 (11.2%)	68 (38%)	0.0141
	Female	2 (0.9%)	23 (9.9%)	32 (13.8%)	106 (45.7%)	

Table 9 Association Between Previous History of Blood Donation and Gender: Do You Think That Your Decision Regarding Blood Donation Can Be Influenced by the Following Factors (1st Not Significant and 2nd iSignificant)?

		You are on any type of medication	Fear of weight gain	You are on a specific diet	You have an active disease	You have received any type of vaccine recently	P-value
Previous history of blood donation	Yes	15 (10.3%)	18 (12.4%)	12 (8.3%)	64 (44.1%)	13 (9%)	0.080
	No	38 (14.3%)	24 (9.0%)	16 (6%)	91 (34.8%)	28 (10.5%)	
Gender	Male	13 (7.3%)	22 (12.3%)	19 (10.6%)	60 (33.3%)	20 (11.2%)	0.002
	Female	40 (17.2%)	20 (8.6%)	9 (3.9%)	95 (40.9%)	21 (9.1%)	

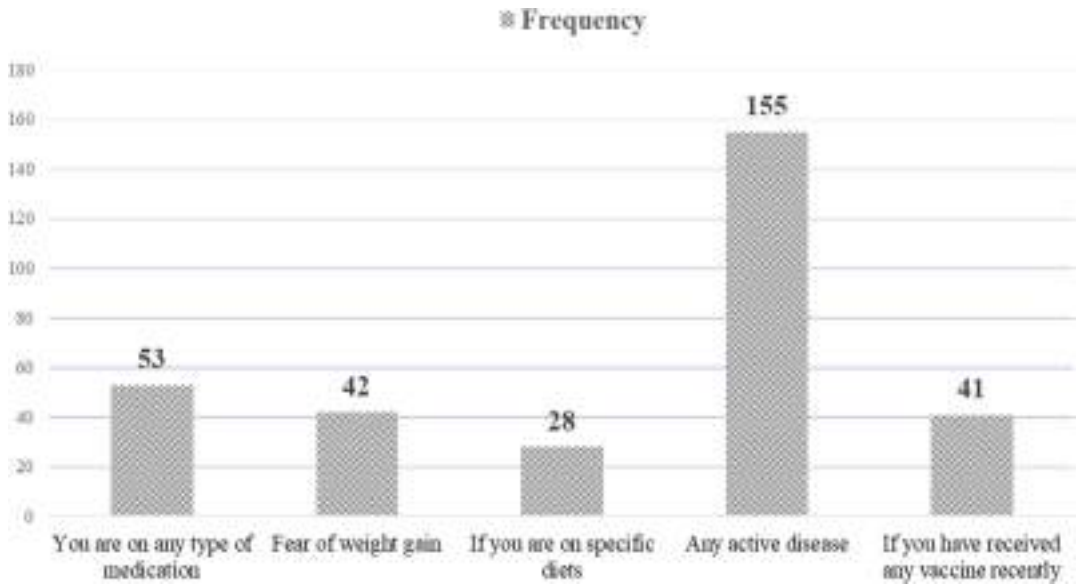

Figure 1 Participant responses to questioning whether their decision regarding blood donation can be influenced.

bottle was another source of fear.[15] Some of these specific fears related to blood are most evident in existing literature, that is, predicting vasovagal reactions and other adverse responses.[16] When compared to other medical treatments that also involve needles, like dental examinations and flu or other vaccinations, people may be less willing to donate blood because it does not positively impact their health.[17]

It was also noteworthy that students from families where blood donation was associated with less medical fear and who had a blood-dependent patient (with thalassemia, for example) and knew the misery they suffered donated blood more regularly than people who experienced a high level of fear related to their general health.[18] Therefore, non-donors can improve their behaviour by first acknowledging their fears and then engaging with specific strategies such as reading material on educational websites and other recruitment materials, engaging in distraction, and making blood donation a normal process, thus boosting their self-confidence.[19] The motivation of non-donors could be boosted by making the donation process less time-consuming, giving them a reward for donating, informing them that blood supplies are low or actually showing them transfusion-dependent patients getting better.[20] Addressing blood-related fears is important for both clinics and promotional campaigns. Donation clinics should be advised to make the environment comfortable, avoid having blood bags and needles within full view, and educate donors with appropriate coping strategies (e.g. distraction or relaxing activities) to overcome their apprehension.[21]

The current findings in our study show that most of the donors were male and have donors in their family. Psychologically, fear reduction would improve attitudes towards blood donation. As a lower economic status country, donations are made by mostly repeat or paid donors. There are far fewer volunteer donors. Promotional campaigns could consider avoiding prompting blood-related fears (e.g. limiting images related to blood on relevant media) and providing some level of reassurance to eligible donors where possible.

The most common reason not to donate blood was fear of general health-related problems, fear of contracting infections because, in Pakistan, there are very few blood centers where all internationally recommended facilities are available. Offering reassurance and facilitating good quality services can increase the number of volunteer donors. Intention to donate is a consequence of attitude and self-efficacy. It is likely that donation history influences attitude, and attitude influences donation. Donors may be able to reconsider their thoughts, feelings, and attitudes. Acknowledging the fears of donors, especially blood drawing and blood-related fears, is critical to develop improved recruitment and retention strategies. Reducing the fears and boosting the self-confidence of potential donors may help improve donation intention. We have to improve our donor management services and maintain them according to international recommendations in order to establish a more stable blood supply.

Conclusion

Our results suggest that most people refrain from donating blood because of various fears and myths such as fear of needle prick, contracting infections, religious issues, health issues, headache, dizziness, weight gain, medications and active diseases. These fears and concerns related to blood donation play a major role in forecasting donor attitude and intention. Motivation leads to inspiration and potential donors can be motivated by addressing their fears.

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COVID-19-Associated Immune Thrombocytopenic Purpura in a Hemodialysis Patient

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Open Access Full Text Article CASE REPORT

COVID-19-Associated Immune Thrombocytopenic Purpura in a Hemodialysis Patient

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Background: Severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection has been a global threat since the end of 2019. Although the main clinical manifestation of coronavirus disease 2019 (COVID-19) is respiratory, its range of clinical manifestation is extensive and may include various systems, including hematological disorders, such as lymphopenia, thrombotic events, thrombocytopenia and immune thrombocytopenic purpura (ITP). The present case was the first one that aimed to raise awareness of ITP induced by COVID-19 in patients undergoing maintenance hemodialysis.

Case Presentation: This is the case of a 75-year-old Asian woman who was diagnosed COVID-19 positive 15 days before attending our Emergency Department on January 19th, 2023, with a three-day history of severe bleeding symptoms, including gastrointestinal, mucosal bleeding, epistaxis, and the platelet count of 5×10^9/L. She suffered from end-stage kidney disease due to autosomal dominant polycystic kidney disease and has received thrice-weekly maintenance hemodialysis (MHD) since 2012. Platelet count recovery was observed after 45 days of combined treatment with corticosteroids, intravenous immunoglobulin, thrombopoietin receptor agonists, and rituximab. The count of platelets rose to 180×10^9/L after four dosages of Rituximab.

Conclusion: In brief, SARS-CoV-2 infection might trigger the onset of ITP. To our knowledge, this is the first case with severe and refractory ITP secondary to COVID-19 in MHD patients and no guidelines were able to be referred on the therapy. Nephrologists must be concerned with clinical characteristics, diagnostic flowcharts, and therapy for SARS-CoV-2-induced ITP.

Keywords: COVID-19, SARS-CoV-2, immune thrombocytopenic purpura, bleeding, platelet

Introduction

Thrombocytopenia is a risk factor for increased morbidity and mortality in patients with the new severe SARS-CoV-2 infection (COVID-19 infection) since the end of 2019.[1] Thrombocytopenia in COVID-19 patients may be caused by viral infections, sepsis, disseminated intravascular coagulation (DIC), or drugs.[2] Recently, several cases reported that (ITP) may be associated with COVID-19 infection and the mechanism may include changes in the bone marrow environment, changes in megakaryocytic differentiation, and maturation resulting from infection.[3,4] Additionally, the correlation mechanism between SARS-CoV-2 and ITP may involve multiple pathways, including direct viral infection of megakaryocytes, which impairs platelet production, and immune-mediated mechanisms where autoantibodies target platelets, leading to their destruction.[1] SARS-CoV-2 infection can also result in an inflammatory environment, with elevated cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) contributing to both platelet destruction and inhibition of megakaryocyte maturation.[5]

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Although the emergence of ITP in patients with COVID-19 is not a novel subject, we firstly present a patient undergoing maintenance hemodialysis (MHD) with COVID-19 infection, with a nadir platelet count of 1×10^9/L and complete response to corticosteroids, intravenous immunoglobulin (IVIG), thrombopoietin receptor agonists (TPO-RAs) and rituximab. Hemodialysis patients are already immunocompromised, which further complicates the management of ITP, as they are at increased risk for both bleeding and thrombotic events. This immune dysregulation, coupled with the inflammatory response to COVID-19, creates unique challenges for ITP management in this population.

Case Description

A 75-year-old Asian woman, who was diagnosed with SARS-CoV-2 positive 15 days before, was admitted to our Emergency Department on January 19th, 2023, with a three-day history of severe bleeding symptoms, including gastrointestinal, mucosal bleeding, and epistaxis. Written informed consent was obtained from the patient, and the study was approved by the institutional review board. She suffered from end-stage kidney disease (ESRD) due to autosomal dominant polycystic kidney disease (ADPKD) and has received thrice-weekly MHD since 2012. The patient had been on long-term hemodialysis and was regularly taking valsartan to control blood pressure. The patient was diagnosed with a femoral neck fracture due to a fall on November 18th, 2021, and the arteriovenous fistula thrombosis occurred on December 5th, 2021. A central venous catheter was placed for subsequent hemodialysis, and the arteriovenous fistula was abandoned. The patient underwent cerebral imaging, which revealed no abnormalities. One week before admission, she received thrombolytic therapy with urokinase for central venous catheter dysfunction, but this was not the first time she had used urokinase. Laboratory evaluation revealed a white blood cell (WBC) of 3×10^9/L, lymphocyte% 10.8%, hemoglobin (Hb) level of 36 g/L, and platelet count of 3×10^9/L. And the RT-PCR test for COVID-19 was negative. Chest radiography revealed no pathological findings. She was treated with a transfusion of red blood cells, platelet units, intravenous immunoglobulin, human recombinant thrombopoietin (rhTPO), and TPO-RAs for several days, but there was no response to the platelet, then she was transferred to our department on February 10th, 2023.

On physical examination, she was pale and afebrile and showed no difficulty breathing in room air with a peripheral oxygen saturation of 95%. Examination of the skin and mucosa showed extensive ecchymosis, especially on both upper extremities. Laboratory evaluation revealed a severe thrombocytopenia of 2×10^9/L, hemoglobin value of Hb 81 g/L, and reticulocytosis of 9.02%, WBC count of 2.8×10^9/L, and a CRP of 9.14 mg/L. Coagulation tests showed D-Dimer was elevated to 2.43 μg/mL and fibrinogen levels were normal. Biochemical tests also showed the following were abnormal: lactate dehydrogenase (LDH) 280 U/L, total bilirubin 35.6 umol/L, direct bilirubin 9.5 umol/L and creatinine 254 umol/L (Table 1). The viral hepatitis panel and rheumatological markers did not reveal any causes of thrombocytopenia. The fecal occult blood test still showed a weak positive. The direct and indirect Coombs tests were both negative. On a peripheral blood smear, there were no schistocytes visible and the platelet count was scarce. ADAMTS 13 activity was 19.1% (68–131). However, platelet autoantibodies and ADAMTS 13 antibodies were negative. Due to the long-term exposure of low molecular weight heparin and unfractionated heparin in hemodialysis sessions before the bleeding symptoms, the heparin-induced thrombocytopenia (HIT) was screened. Anti-platelet factor 4 (PF-4)/heparin antibodies or functional assays were negative. Bone marrow biopsy was performed after the platelet count rose and showed active thrombopoiesis. All relevant medications (valsartan, urokinase, erythropoietin, calcitriol) were checked to rule out drug-induced thrombocytopenia.

Since severe thrombocytopenia carried a high risk of fatal bleeding in hemodialysis, although the exact mechanism of thrombocytopenia remained unclear, we speculated it might be immune-related because other causes of thrombocytopenia including DIC, thrombotic thrombocytopenic purpura with no hemolytic anemia, HIT and sepsis-induced thrombocytopenia had been excluded. So the patient was treated with a low dose of methylprednisolone (40 mg daily), IVIG (20 g daily for 5 days), and platelet transfusion (1 unit daily for 5 days) on February 10th, along with other protection strategies, such as PPI, albumin, erythropoietin, folic acid, and VitB12 supplement (Table 2). We stopped the transfusion of platelet units because of no bleeding events and the stable hemoglobin level. Nafamostat mesylate 20mg/h was chosen as the anticoagulant to prevent additional consumption of the platelet during hemodialysis. However, no response of platelet count was observed. By the 8th day after admission to our department, her platelet count was still only 5×10^9/L, and her Hb level rose to 96 g/L. Since February 22nd, the dexamethasone (20 mg daily for 3 days) and the second-line

Table 1 Laboratory Changes During This Case

Laboratory Data	Normal Range	Day1	Day23	Day53	Day61	Day65	Day69
WBC	$3.5–9.5\times10^9$/L	3	2.8	3.8	10.3	9	6.4
Neutrophils	40–75%	73.7	63.8	70	86.8	88.2	86
Lymphocytes	20–50%	10.8	17.4	17	18.6	16.8	18.7
RBC	$3.8–5.1\times10^{12}$/L	1.26	2.55	3.3	3.38	3.34	3.59
Hemoglobin	115–150g/L	36	81	104	100	97	102
Platelet	$125–350\times10^9$/L	3	2	57	180	221	304
CRP	0–10mg/L	13.14	9.14	22.42	51.19	62.41	40
Total bilirubin	3.4–17.1μmol/L	18.4	35.6	16.2	21	18.6	17.2
Direct bilirubin	0–3.4μmol/L	0	9.5	3.3	4.2	5	5.4
AST	7–40U/L	6	2	9	9	4	2
ALT	13–36U/L	13	19	11	11	9	14
LDH	120–250U/L	290	280	203	211	184	180
BUN	2.6–8.8mmol/L	32.3	6.6	23.8	21.6	16.3	20.8
Serum creatinine	41–81μmol/L	646	254	363	372	386	378
Fibrinogen	2–4.5g/L	3.08	2.09	3.55	2.89	1.04	2.89
D-dimers	0–1.0μg/mL	3.7	3.42	2.39	1.9	4.83	1.9
FDP	<5.0mg/L	12	8.6	8.8	6.2	14.3	6.2
PCT	<0.5ng/mL	-	0.433	0.584	-	1.36	1.18
IL-6	<7pg/mL	-	59.25	55.4	-	400.5	101.6

Abbreviations: WBC, white blood cells; RBC, red blood cells; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; FDP, fibrin degradation product; PCT, procalcitonin.

Table 2 Medication History and Treatment Strategies

Medication/Treatment	Indication	Outcome
Valsartan	Hypertension control	Stable blood pressure
Urokinase	Thrombolytic therapy (AVF)	No adverse effects; used multiple times
Methylprednisolone	Initial ITP treatment	No significant improvement in platelet count
IVIG	Second-line therapy for ITP	No response after 5 days of treatment
Platelet transfusions	Bleeding symptoms	No sustained improvement in platelet count
Eltrombopag	TPO-RA, second-line therapy	Platelet count started to rise
Rituximab	Refractory ITP treatment	Significant increase in platelets after 4 doses

treatment of eltrombopag (50 mg daily for 28 days), rhTPO (15000U for 30 days), and Rituximab (100 mg once weekly for 4 doses) were used for treatment. The count of platelets rose to 180×10^9/L after four dosages of Rituximab until March 20th and reached 304×10^9/L on March 28th (Figure 1). The patient was discharged after her platelet count normalized, and she exhibited no signs of bleeding. She continues to visit our dialysis center three times a week, where her platelet count is regularly monitored as part of her follow-up care. The excellent prognosis after immunosuppressive therapy was observed until 1.5 months later upon admission to our department, which confirmed the immune mechanism mediated thrombocytopenia.

Discussion

To our knowledge, this is the first case with severe and refractory ITP secondary to COVID-19 in MHD patients and no guidelines were able to be referred on the therapy. ITP is an immune-mediated acquired hemorrhagic disorder with a low platelet count characterized by increased peripheral platelet destruction and insufficient platelet production.[6] ITP incidence has been estimated to be from 1.6–3.9/100,000 person-years in adults,[1] and the incidence of ITP in COVID-19 patients is estimated to be rarer. While most cases are mild, severe thrombocytopenia can occur in 10–20% of cases, with a higher risk of mortality in patients with comorbidities, such as ESRD or those undergoing hemodialysis.[7] Thrombocytopenia in COVID-19 can be attributed to various mechanisms, including direct viral infection of bone

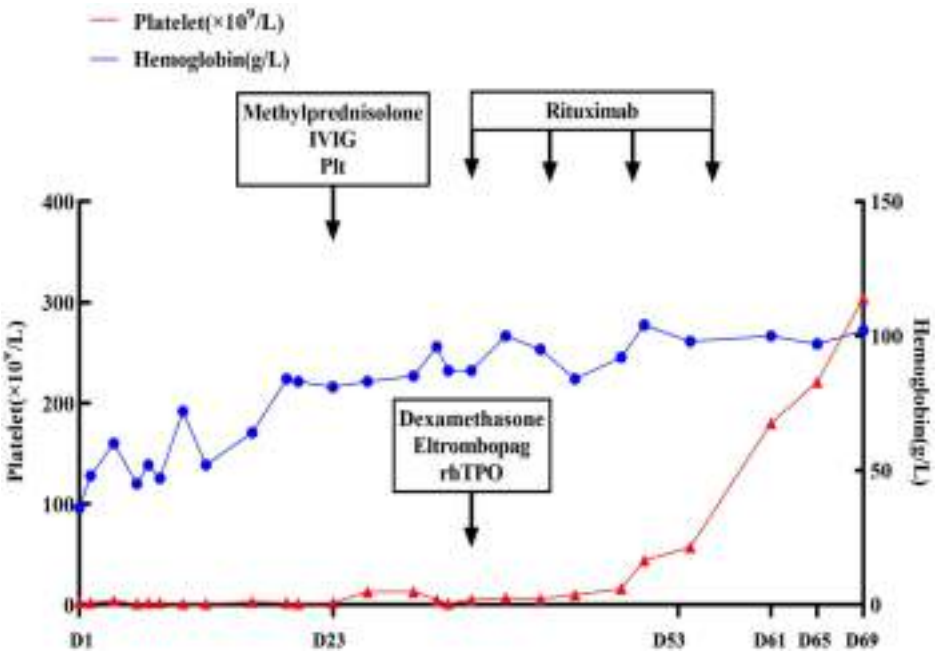

Figure 1 The trends of the patient's platelet and hemoglobin count.

marrow megakaryocytes, the cells responsible for platelet production. The inflammatory milieu, characterized by elevated levels of cytokines such as IL-6 and TNF-α can contribute to platelet destruction and impaired megakaryocyte function.[8] For most patients, the mechanism is mediated by the antiplatelet antibodies secreted by plasma cells linked to the platelet surface glycoproteins GPIb/IX and GPIIb/IIIa, leading to the clearance of platelets. Moreover, antibodies linked to megakaryocytes can inhibit their maturation and can trigger their destruction. However, up to 30–40% of ITP patients show negative antibodies. So in most cases, it is an exclusive diagnosis based on clinical judgment and independent of antibody status. The proposed causes include viral infections, changes in the bone marrow environment, changes in megakaryocytic differentiation and maturation, abnormal T cells, imbalance in cytokine secretion, and immune dysregulation.[9]

Thorough evaluation is crucial to differentiate COVID-19-induced ITP from idiopathic ITP. This may involve ruling out other causes of thrombocytopenia, such as drug-induced, sepsis-associated, or DIC-related thrombocytopenia. ITP has emerged as a complication after COVID-19 vaccination and infection. The incidence of ITP secondary to COVID-19 infection is more common among males (54.8%) and it is more prevalent among the elderly with a median age of 63 years.[2] COVID-19-induced ITP occurred within 2–3 weeks with an estimated mean 18.1±21 days after SARS-CoV-2 infection and in most cases presented with asymptomatic mild and moderate thrombocytopenia. Severe and persistent thrombocytopenia, like in our case, was seldom reported. The possible thrombocytopenia pathogenic mechanisms such as molecular mimicry, cryptic antigen expression, or epitope spreading depend on the phase of COVID-19.[10]

Usually, the first-line therapy for ITP treatment aims to get a rapid response and increase the platelet count, especially for those patients with active bleeding. Corticosteroids remain the initial treatment and prednisone (0.5–2.0 mg/kg per day) or dexamethasone (40 mg per day for 4 days) are preferred, which can reduce the inflammation and autoimmunity. IVIG is another first-line treatment option for patients at risk of serious bleeding since it can provide passive immunity and modulate the immune system by neutralizing autoantibodies and inhibiting complement deposition. IVIG should be administrated at a dose of 400 mg/Kg/day for 5 days or 1 g/kg for 1–3 days, which could be combined with or without corticosteroids. Rituximab was used for ITP for the first time in 2001. The rationale for its use in refractory cases by explaining its mechanism of depleting CD20-positive B cells, which are implicated in the pathogenesis of autoantibody-mediated conditions like ITP.[11] The dosage of rituximab of 375 mg/m^2 and lower 100 mg were both reported in several studies.[12] TPO-RAs, whose effectiveness in stimulating platelet production in the bone marrow is highlighted, are particularly pertinent in cases where thrombocytopenia persists despite control of the

underlying disease process. Leading to a sustained increase in platelet count 1–2 weeks after treatment. However, it also increases the risk of venous thromboembolism and hepatotoxicity, recommendations suggest using TPO-RAs as a second-line treatment.[13] RhTPO is also used in China and has shown efficiency in ITP. Moreover, hemodialysis patients are at increased risk of infections and complications due to their immunocompromised status. Monitoring for complications, including infection and coagulation function disorders like hemorrhage, is crucial in this population.[7]

The management of COVID-19-associated ITP in hemodialysis patients presents unique challenges. These patients are more vulnerable to infections and have an increased thrombotic risk due to both the nature of ESRD and the treatments used for ITP, such as corticosteroids and TPO receptor agonists. The uniqueness of this case lies in the difficulty of managing refractory ITP in a patient with ESRD on long-term hemodialysis, in the context of SARS-CoV-2 infection, without the usual COVID-19 respiratory complications. The absence of established guidelines for treating ITP in this specific population adds to the complexity.

This case report has several limitations. First, although drug-induced thrombocytopenia was considered and evaluated, we cannot completely exclude the possibility of unreported medications or supplements contributing to thrombocytopenia. Second, the patient's comorbid conditions, apart from ADPKD and ESRD, were not thoroughly investigated due to the urgency of her presentation, which could have influenced the clinical outcome. Third, given the rarity of COVID-19-induced ITP in MHD patients, the generalizability of this case may be limited. Further studies and case reports are necessary to better understand the pathophysiological mechanisms and optimal treatment strategies for ITP in this specific population. Finally, the absence of established guidelines for the management of COVID-19-associated ITP in MHD patients limited our ability to follow standardized treatment protocols, resulting in a reliance on clinical judgment and available therapeutic options.

In brief, SARS-CoV-2 infection might trigger the onset of ITP. Thrombocytopenia in the context of COVID-19 presents unique challenges, especially in vulnerable populations like hemodialysis patients. The development of ITP following COVID-19, although rare, should be considered in cases of persistent thrombocytopenia. Timely diagnosis, appropriate immunosuppressive therapy, and close monitoring are essential for achieving optimal outcomes in these patients. Further research is needed to elucidate the pathophysiological mechanisms underlying COVID-19-associated thrombocytopenia and its potential role in triggering immune-mediated disorders like ITP. The SARS-CoV-2 Omicron variant is still prevalent in hemodialysis populations with poor prognosis worldwide. Nephrologists must be concerned with clinical characteristics, diagnostic flowcharts, and therapy for SARS-CoV-2-induced ITP.

Abbreviations

WBC, white blood cells; RBC, red blood cells; Ne, neutrophils; Ly, lymphocytes; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; FDP, fibrin degradation product; PCT, procalcitonin; ITP, immune thrombocytopenia; MHD, maintenance hemodialysis; IVIG, intravenous immunoglobulin; TPO-RAs, thrombopoietin receptor agonists; ESRD, end-stage kidney disease; HIT, heparin-induced thrombocytopenia; ADPKD, autosomal dominant polycystic kidney disease, DIC, disseminated intravascular coagulation.

Data Sharing Statement

All data collected from the patient were obtained from Changzheng Hospital and were available in this paper.

Ethics Statement

The patient received all information regarding this case report. Written informed consent for publication in Journal of Blood Medicine was obtained from the patient.

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Disclosure

The authors declare that they have no competing interests.

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Thrombotic Risk Assessment, P-Selectin, and Thromboprophylaxis Use Among, Cancer Patients at the University of Calabar Teaching Hospital, Calabar

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Open Access Full Text Article ORIGINAL RESEARCH

Thrombotic Risk Assessment, P-Selectin, and Thromboprophylaxis Use Among, Cancer Patients at the University of Calabar Teaching Hospital, Calabar

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Background: Venous thromboembolism is the second leading cause of mortality among cancer patients. The Khorana Risk Assessment Score (KRAS) is widely acknowledged as the most validated tool in this context.

Aim: To assess the thrombotic risk in cancer patients using the modified Khorana Risk Assessment Score, examine the association between modified KRAS and soluble P-selectin levels, and document the utilization of thromboprophylaxis among cancer patients at the University of Calabar Teaching Hospital.

Methods: This was a cross-sectional hospital-based recruiting 100 cancer patients. Seven millilitres of blood were collected for complete blood count and P-selectin assay. Continuous variables were expressed as mean and standard deviation, while categorical variables were summarized using frequencies. Chi-square was employed to compare VTE risk status across genders, different cancer types, and guideline compliance. The significance level was set at 0.05.

Results: Participants age ranged from 19 to 87 years, with a male-to-female ratio of 1:1.6. The most common female cancer was Breast at 40.32% and prostate cancer at 65.79% was the most common in males. Seventy nine percent and 21% of participants had intermediate and high-risk modified KRAS scores respectively. The median level of soluble P-selectin among cancer patients was 23.00 within the interquartile range. Significant associations were observed between cancer types and sex, VTE risk assessment and cancer types, and cancer types and risk score.

Conclusion: The risk of VTE among cancer patients ranges from intermediate to high, going by the modified Khorana risk score irrespective of the P selectin level, with underutilization of thromboprophylaxis. There is little adherence to the Khorana score in our setting, hence the need for greater application and knowledge of this predictive score in clinical practice to improve outcomes and quality of life.

Keywords: thrombosis, cancer, P-selectin

Introduction

The incidence of cancer is rising significantly with a global estimate of 18.1 million and 9.6 million deaths. Over 600,000 deaths occur annually in Africa.[1] It was estimated that by the end of 2020, the incidence of cancer in Africa will rise to about 15 million.[2] The prevalence of cancer in Nigeria is estimated at 211,052 with about 70,327 deaths and 115,950 new cases.[1] In Cross River State, Ebughe et al reported a total of 941 cancer patients at the University of Calabar in a ten years study.[3] Venous thromboembolism (VTE) which is comprised of pulmonary embolism (PE) and deep vein thrombosis (DVT), is one of the leading causes of mortality among cancer patients. The prognostic implications of VTE in cancer have been studied in different types of cancer.[4–7] Available data suggests that VTE in malignancies is associated with tumour aggressiveness and can adversely impact the survival of patients.[5,8] Cancer is known as a hypercoagulable and prothrombotic disease associated with significant

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alteration of the haemostatic system. This is due to the release of tissue factor and procoagulant molecules by the neoplasm.[5] There is also increased expression of activated factor X, increased expression of TF, plasminogen activator inhibitor-1 (PAI- 1), urokinase plasminogen, and tissue-type plasminogen activator with prothrombotic potential. Cancer cells secrete a lot of cytokines TNF-α and IL-1β, which causes expression of TF and down-regulates thrombomodulin predisposes to hypercoagulability and risk of VTE.[4–7] Other reported mechanisms for hypercoagulability include angiogenesis, the mechanical effect exerted by the tumours, and increased endothelial cell activation among others.[9] Besides cancer, other VTE-associated risk factors include surgery, trauma, hospitalization, cancer with or without chemotherapy, and also the use of central nervous catheters. Risk assessment models (RAM) such as the Khorana score can be used to predict the risk of VTE in cancer patients. The Khorana Risk Score (KRS) is a tool that was introduced in 2008 and was designed to predict the risk of VTE in cancer patients based on the type of cancer, body mass index, and haematological parameters of the patient. It stratifies patients into no risk, low and high risk of VTE, and therefore forms the basis for recommending the use of anticoagulants (either thromboprophylaxis or antithrombotic therapy). KRS score is endorsed by the American Society of Clinical Oncology (ASCO), the International Society for Thrombosis and Haemostasis (ISTH), and the National Comprehensive Cancer Network (NCCN). KRS assigns points to five clinical and pre-chemotherapy laboratory parameters. The parameters include; primary tumour site, platelet count, haemoglobin concentration, or use of erythropoiesis-stimulating agents, leukocytes count, and body mass index. Patients with a KRS of greater than 3 are considered at high risk for developing blood clots and may benefit from prophylactic anticoagulation. In addition to the use of risk assessment models, the use of biomarkers has been suggested to increase the sensitivity of predicting VTE in at-risk patient groups[10] One such molecule is P-selectin. P-selectin is a transmembrane protein present in the α granules of platelets and the Weibel-Palade bodies of endothelial cells, during activation, it is rapidly translocated to the cell surface. P-selectin mediates the rolling of platelets and leukocytes on activated endothelial cells as well as interactions of platelets with leukocytes[11] The aim of this study is to assess the risk pattern of VTE, practice of thromboprophylaxis and correlate the level of P-selectin as a biomarker for thrombosis and VTE risk score using KRS among cancer patients.

Methods

Study Area

The study was carried out in Cross River State specifically at the University of Calabar Teaching Hospital (UCTH). UCTH is a Federal Government-owned tertiary institution, situated in Calabar Municipality LGA, Calabar, and Cross River State. The facility receives referrals from neighbouring states such as Akwa Ibom, Bayelsa, Ebonyi, and Rivers States.

Study Design

This was a cross-sectional study. This was deemed appropriate for this research because it was an observational study involving cancer patients seen at the University of Calabar Teaching Hospital and clinicians who manage cancer patients. The vegetation ranges from mangrove swamps, through rainforest, to derived savannah, and montane parkland.

Study Population

This study involved all cancer patients seen at the University of Calabar Teaching Hospital and clinicians who manage cancer patients.

Inclusion Criteria

The study population consisted of

1. Adult cancer patients (≥18 years) receiving care at the University of Calabar Teaching Hospital who met the study inclusion criteria;
2. Primary or relapsing cancer patients;
3. Planned initiation of cancer therapy (chemotherapy, hormone therapy, molecular targeted therapy, immunotherapy, radiation therapy, or surgery); or
4. Planned first-line therapy for patients with relapsed cancer; provision of informed consent.

Exclusion Criteria

1. Patients with only intramucosal cancer,
2. Brain cancer,
3. Bleeding disorders, other
4. Contrary indication for thromboprophylaxis, and
5. Those judged as inappropriate for inclusion or difficult to follow up by the investigators were excluded.

Sample Size Estimation

The sample size of 100 after factoring in 10% attrition was calculated using the Kish Leslie formula with a reference proportion of 6.4% from a study by Ay (Ay et al, 2008)

Sampling Technique

The researchers employed a purposive sampling technique, a non-probability sampling method used when the researcher aims to study a specific group of individuals or phenomena based on their unique characteristics and relevance to the research. Target patients with histological evidence of cancer were recruited from various clinics (Surgical, Medical, and Haematology Clinics) involved in the care of cancer patients.

Study Tools

A workshop on thrombotic risk assessment was carried out in all the clinical units involved in the management of cancer patients to educate them on the guidelines for thrombotic risk assessment in cancer patients and how to use the Khorana Risk Score. The patient information leaflet was also designed to educate cancer patients on their risk of thrombosis and the benefits of thromboprophylaxis. Recruited subjects were evaluated for thrombotic risk using the Khorana risk scoring tool (Table 1) and a blood sample collected for evaluation of p selectin levels.

Pre-Testing

Basic Haematological Parameters involved using full blood count which includes haematocrit, haemoglobin concentration, and total white cell and platelet counts were obtained from the EDTA sample, using an automated blood cell counter (Sysmex Haematology Auto- analyser model KN21, Texas, USA). The basic principles underlying this technique are electronic impedance and light scattering. This was done in the main Haematology Laboratory, UCTH. In the P-selectin

Table 1 Khorana Risk Scoring Model Table

Patient Characteristics	Risk Score Points
Cancer Type	
Stomach (Very high risk)	2
Pancreas (Very high risk)	2
Primary brain tumour (Very high risk)	2
Lung	1
Lymphoma	1
Gynaecologic	1
Bladder	1
Testicular	1
Renal	1
Pre-chemotherapy platelet count ≥ $350x10^9$/L	1
Haemoglobin level <10g/Dl or using RBC growth factors	1
Pre-chemotherapy leukocyte count > $11x10^9$/L	1
Body Mass Index ≥ $35kg/m^2$	1

Notes: low risk= (0 points), intermediate-risk (1–2 points), high risk (≥3 points).

assay, the samples of patients with suspected DVT were screened further using the P-selectin assay. An immunological assay based on an enzyme-linked immunoassay was used.

Statistical Analysis

The data was collected and analyzed using the Statistical Package for Social Sciences version 26. Data of continuous variables were expressed as mean, standard deviation and categorical variables will be summarized using frequencies. Chi-square was used to compare VTE risk status between males and females, across different cancer types, and between those who complied with guidelines. P-value was set at ≤ 0.05.

Ethical Approval

The study obtained ethical clearance from the University of Calabar Teaching Hospital, Nigeria under number HREC 41285 and all participants provided informed consent. The study complies with the Declaration of Helsinki.

Results

Description of Sociodemographic Characteristics

The sample population consisted of 100 subjects, spanning various age groups. The largest age group, comprising 44% of the subjects, was individuals aged 40–59, followed by those aged 60–79 (30%). Participants aged 20–39 made up 21%, while those aged 0–19 and 80+ represented 1% and 4%, respectively.

For gender distribution, females constituted 62% of the sample, with males making up 38%. Most participants were married (73%), with single individuals accounting for 21%. A smaller proportion were widowed (5%) or separated (1%).

Educational backgrounds varied, with the largest segment having completed secondary education (39%), followed by those with tertiary education (37%). Primary education holders accounted for 15%, and 9% were uneducated.

The majority of participants (72%) had experienced their illness for 0–2 years, while 22% had been ill for 3–5 years. Only 6% had a duration of illness exceeding five years. A comprehensive overview of the socio-demographic characteristics of the patients is provided in Table 2.

Cancer Types

Figure 1 displays the distribution of cancer types among the participants. The most prevalent types were breast and prostate cancers, each with 25 cases, accounting for 25% of the total. Ovarian cancer followed, with 13 cases (13%), and lymphoma, with 12 cases (12%). Leukemia was found in 6 cases (6%), while cervical cancer appeared in 3 cases (3%) and vulvar cancer in another 3 cases (3%). Bladder, endometrial, lung, Meigs syndrome, and pancreatic cancers each had 2 cases (2%). Less frequent types, each representing 1% of the sample, included gastric cancer, glioblastoma, and osteosarcoma.

Year of Diagnosis

Figure 2 details the distribution of cancer diagnoses by year. Diagnoses were minimal from 2007 to 2016, with just one case per year (1% each). Diagnoses began to rise in 2017 and 2018, with five (5%) and seven cases (7%), respectively. This trend continued in 2019 (5%) and 2020 (8%). The number of diagnoses peaked in 2021, with 13 cases (13%), and surged in 2022, when 59 cases were recorded, accounting for 59% of the total sample.

Treatment Plan

Table 3 summarizes the treatment modalities used within the sample population. Chemotherapy was administered to 28% of participants, while 72% did not receive it. Surgery was performed on 45% of the sample, with 55% not undergoing surgery. Radiation therapy was utilized by 49% of participants, while 51% did not receive it. Only 4% of the sample received anticoagulant prophylaxis, with the remaining 96% not receiving this treatment option.

Table 2 Demographic Characteristics

Demography	Frequency (n=100)	Percentage (%)
Age range in years		
0–19	1	1.00
20–39	21	21.00
40–59	44	44.00
60–79	30	30.00
≥ 80	4	4.00
Gender		
Male	38	38.00
Female	62	62.00
Marital Status		
Married	73	73.00
Single	21	21.00
Widowed	5	5.00
Separated	1	1.00
Education		
Primary	15	15.00
Secondary	39	39.00
Tertiary	37	37.00
Uneducated	9	9.00
Duration of Illness (years)		
0–2	72	72.00
3–5	22	22.00
>5	6	6.00

Association Between Type of Cancer and Sex Distribution

As shown in Table 4; among males, prostate cancer was the most common, comprising 65.79% of male cases, followed by lymphoma (13.16%) and smaller percentages of leukaemia, bladder, gastric, glioblastoma, and lung cancers. In females, breast cancer had the highest frequency at 40.32%, with ovarian cancer at 24.19%, followed by lymphoma,

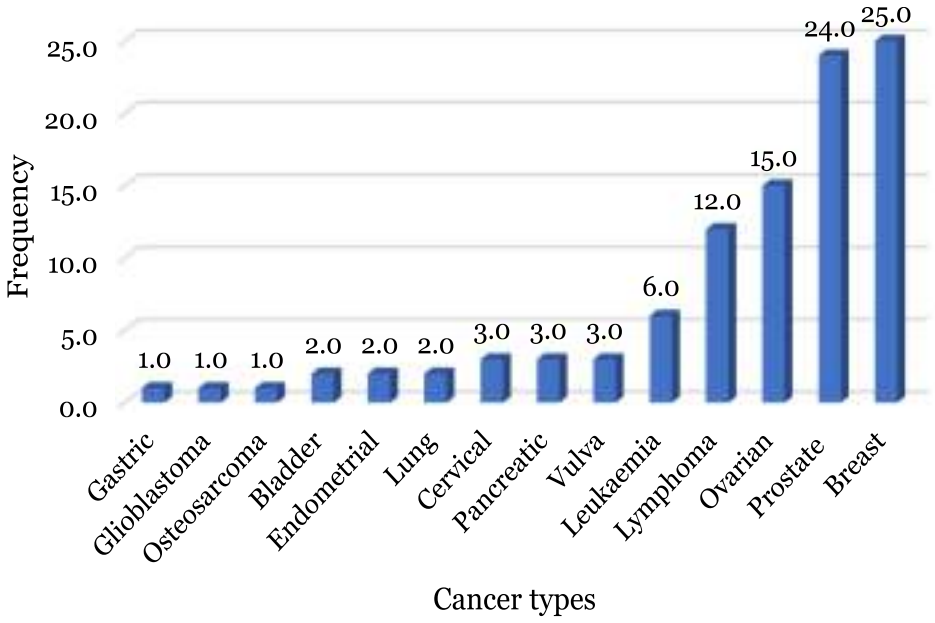

Figure 1 Different Cancers of the subjects.

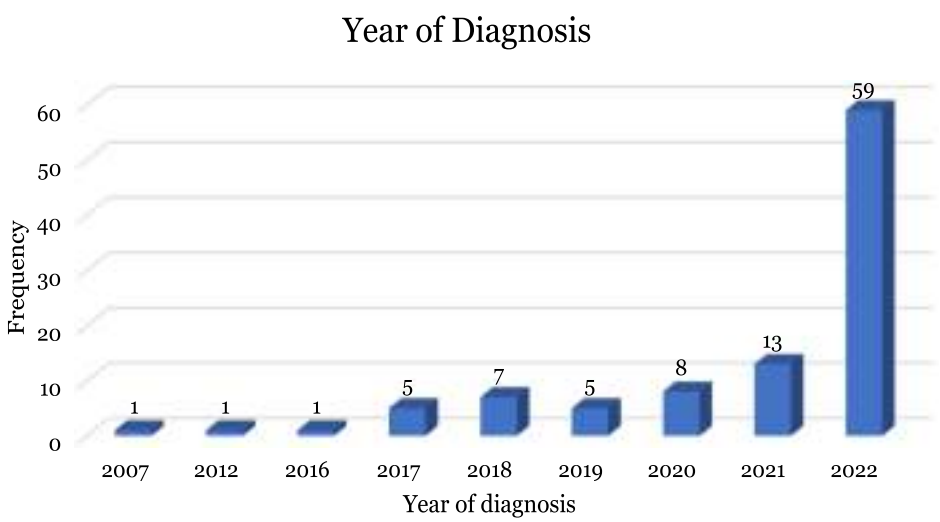

Figure 2 Years of Disease Diagnosis.

cervical cancer, and leukaemia. A Chi-square test showed a significant association between gender and cancer type (X^2 = 79.131, $p < 0.001$), indicating gender-specific cancer distribution (Table 4).

Blood Count Parameters in the Study Population

Table 5 outlines haematological parameters for the study population, including haemoglobin, white blood cell, and platelet counts. The average haemoglobin level was 11.13 g/dL (SD = 5.74), ranging from 3.80 to 27.20 g/dL, showing considerable variation. The mean white blood cell count was 11.05 x 10^9/L (SD = 21.75), with a broad range from 1.00 to 129.00 x 10^9/L. Platelet counts averaged 189.51 x 10^9/L (SD = 137.87), ranging from 8.00 to 578.00 x 10^9/L, indicating substantial diversity in blood count parameters among participants.

Body Mass Index

Table 6 indicates that the majority of the study population (54%) had a BMI within the "Normal weight" range. An additional 38% were classified as "Overweight", while a smaller proportion (8%) fell into the "Obesity" category, reflecting a predominantly normal to slightly elevated BMI distribution within the sample.

Table 3 Treatment Plan

Treatment	Frequency (n=100)	Percentage (%)
Chemotherapy		
Yes	28	28.00
No	72	72.00
Surgery		
Yes	45	45.00
No	55	55.00
Radiation therapy		
Yes	49	49.00
No	51	51.00
Anticoagulant Prophylaxis		
Yes	4	4.00
No	96	96.00

Note: Source: Researcher's Fieldwork, 2023.

Table 4 Association Between Type of Cancer and Sex Distribution

Sex	Type of Cancer	Frequency	Chi square
Male	Bladder	2 (5.26)	X^2 = 79.131
	Gastric	1 (2.63)	P=<0.001
	Glioblastoma	1 (2.63)	
	Leukaemia	3 (7.89)	
	Lung	1 (2.63)	
	Lymphoma	5 (13.16)	
	Prostate	25 (65.79)	
	Total	38 (100.00)	
Female	Breast	25 (40.32)	
	Cervical cancer	3 (4.84)	
	Endometrial	2 (3.23)	
	Leukaemia	3 (4.84)	
	Lung	1 (1.61)	
	Lymphoma	7 (11.29)	
	Osteosarcoma	1 (1.61)	
	Ovarian	15 (24.19)	
	Pancreatic	2 (3.23)	
	Vulva	3 (4.84)	
	Total	62 (100.00)	

Note: Source: Researcher's Fieldwork, 2023.

Table 5 Blood Count Parameters in the Study Population

Blood Parameters	Values
Haemoglobin (g/dL)	
Mean ± SD	11.13 ± 5.74
Range	3.80–27.20
WBC (x 10^9/L)	
Mean ± SD	11.05 ± 21.75
Range	1.00–129.00
Platelet (x 10^9/L)	
Mean ± SD	189.51 ± 137.87
Range	8.00–578.00

Note: Source: Researcher's Fieldwork, 2023.

Table 6 Body Mass Index

BMI (Kg/m^2)	Category	Frequency	Percentage
<18.54	Underweight	0	0.00
18.5–24.49	Normal weight	54	54.00
25–29.90	Overweight	38	38.00
≥ 30	Obesity	8	8.00

Note: Source: Researcher's Fieldwork, 2023.

P-Selectin Levels (Ng/Ml)

The average P-selectin level among study participants was 23.54 ng/mL (SD = 13.29), with a median of 23.00 ng/mL. The interquartile range (IQR) for P-selectin was 13.00 to 23.00 ng/mL. Presented in Table 7 and Table 8 respectively.

Table 7 P-Selectin Levels in Study Subjects

P-selectin	Values (ng/mL)
Mean ± SD	23.54 ± 13.28
Median	23.00
IQR	13.00–23.00 (20.00)

Note: Source: Researcher's Fieldwork, 2023.

Table 8 Association Between Cancer Types and P-Selectin

Cancer Types	0–9	10–19	20–29	30–39	40–49	50–59	≥60	Total	Statistic
Bladder	0	2	0	0	0	0	0	2	
Breast	2	8	1	12	2	0	0	25	
Cervical	0	0	2	0	1	0	0	3	X^2 = 174.371
Endometrial	2	0	0	0	0	0	0	2	P=<0.001
Gastric	0	0	1	0	0	0	0	1	
Glioblastoma	1	0	0	0	0	0	0	1	
Leukaemia	0	2	2	0	2	0	0	6	
Lung	0	0	2	0	0	0	0	2	
Lymphoma	2	6	4	0	0	0	0	12	
Osteosarcoma	0	0	1	0	0	0	0	1	
Ovarian	4	0	5	2	2	2	0	15	
Pancreatic	2	0	0	0	0	0	0	2	
Prostate	1	6	13	2	3	0	0	25	
Vulva	0	0	1	0	0	0	2	3	
Total	14	24	32	16	10	2	2	100	

Note: Source: Researcher's Fieldwork, 2023.

Khorana Scoring System

In the study, VTE risk scores varied, with the majority (41%) of individuals scoring 1.0, indicating a moderate VTE risk. Another 32% had a score of 2.0, also suggesting moderate risk. Higher risk scores included 3.0 (15% of participants) and 4.0 (5%), associated with elevated VTE risk. Seven individuals had a 0.0 score (7%), representing the lowest risk. The average Khorana risk score was 1.84 (SD = 0.95), with an interquartile range of 1.00 to 2.00, covering moderate risk levels. The findings are presented in Table 9

VTE Risk Assessment

Table 10 reveals that most of the study population (79%) were classified as "Intermediate Risk" for Venous Thromboembolism (VTE), indicating a moderate risk level. A smaller group, representing 21% of participants, fell into the "High Risk" category, suggesting a heightened susceptibility to VTE in this subset.

Association Between VTE Risk and Sex

Table 11 provides a comprehensive analysis of the association between VTE (Venous Thromboembolism) risk assessment and sex within the study population. The independent sample *t*-test was conducted to assess the association between VTE risk assessments and sex. No observable statistical difference was seen in the association between VTE risk and the sex of the patients (t= −0.717, p=0.475).

Table 9 Khorana Scoring System

Risk Score	Frequency	Percentage
0.00	0	0.00
1.00	44	41.00
2.00	35	32.00
3.00	16	15.00
4.00	5	5.00
Mean ± SD	1.84 ± 0.95	
IQR	1.00–2.00 (1.00)	

Table 10 VTE Risk Assessment

Risk Assessment Range	Frequency	Percentage
Intermediate risk (1–2)	79	79.00
High Risk (≥3)	21	21.00

Note: Source: Researcher's Fieldwork, 2023.

Table 11 Association Between VTE Risk and Sex

Sex	VTE Assessment Score		t	P-value
	Mean	SD		
Male	1.71	1.01	−0.717	0.475
Female	1.85	0.96		

Correlation Between P-Selectin and VTE Risk

Correlation between P-selectin and KRS showed a weak negative association (r =−0.08) and the p-value indicates insignificance (p=0.404) as shown in Figure 3.

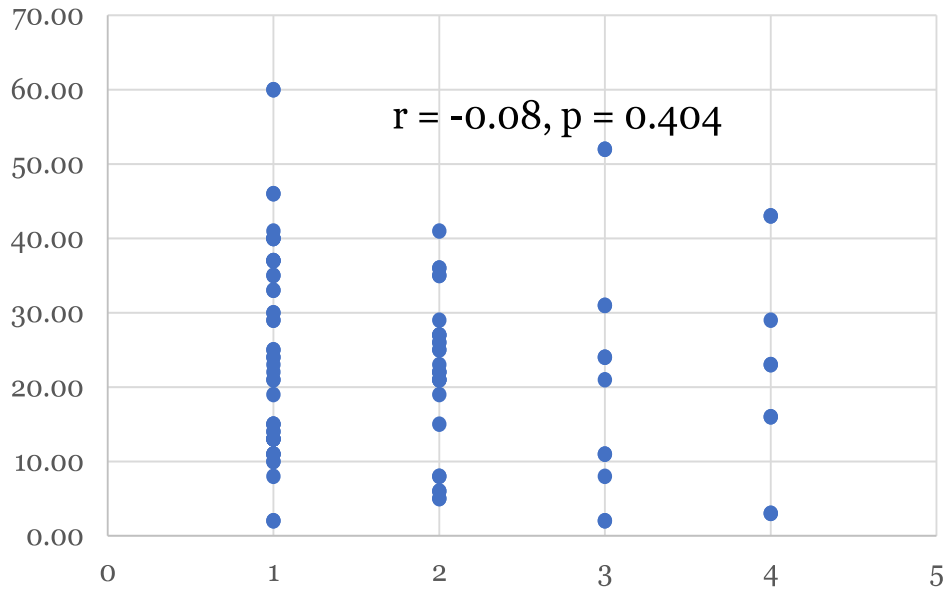

Figure 3 Correlation between P-selectin and KRS.

Discussion

Cancer is a hypercoagulable and prothrombotic disease associated with significant alterations in the haemostatic system. In this study, subjects with cancer were predominantly middle-aged females, with a peak age range of 40–59 years. Most were married, had tertiary education, and had a duration of illness of approximately two years. This demographic trend may be attributed to the health-seeking behaviours of married women and their educational backgrounds. Additionally, a significant portion of our subjects were gynaecological cancer patients, a finding also reported by Omosun et al in Lagos, Southwest Nigeria.[12]

In our study, breast and ovarian cancers were the predominant cancers among females, while prostate cancer was most prevalent among males. This aligns with the findings of Agba et al in North Central Nigeria,[13] who reported a higher prevalence of 66.4% of breast and prostate cancers among females and males, respectively. A similar trend was noted in a study by Uchendu in the South-South region of Nigeria, which found a predominance of breast cancer in females and colorectal cancer in males.[14] In contrast, Omosun et al found a predominance of cervical cancer in the Southwest of Nigeria. These discrepancies may be attributed to variations in study design.[12]

Our study demonstrated that surgery was the most common treatment option, followed by radiation and chemotherapy, with anticoagulant use recorded at 4%. This finding is consistent with the work of Cai et al, who investigated thromboprophylaxis for inpatients with advanced cancer in a palliative care setting.[15] Additionally, Mahan et al reported a thromboprophylaxis rate of 3.9% among medically ill patients, which mirrors our findings.[16]

The haematological parameters varied significantly in our study; haemoglobin levels ranged from severe anaemia to polycythaemia, while white blood cell counts varied from severe leukopenia to marked leucocytosis. Platelet counts also exhibited a range from severe thrombocytopenia to moderate thrombocytosis. These variations can be attributed to the effects of chemotherapy and the different stages of cancer, as well as the potential impact of systemic inflammation related to cancer.[17,18]

Our findings indicate that most subjects had a normal BMI, with few classified as overweight and even fewer as obese. This is in line with the findings of Fadelu et al, who also reported normal BMI in a study of body mass index, chemotherapy-related weight changes, and disease-free survival in Haitian women. This similarity may be due to the predominance of breast cancer among our subjects and the chronicity of the diseases.[19] In contrast, Pati's study indicated an association between obesity and breast, endometrial, and pancreatic cancers.[20]

The soluble P-selectin levels in our subjects were found to be within the median interquartile range. Soluble P-selectin, which is released by activated platelets, is associated with thrombosis risk. This finding aligns with Ayc et al,[21] who noted no change in soluble P-selectin levels in newly diagnosed cancer patients, though these levels were associated with venous thromboembolism risk. Similarly, Castellon et al reported normal P-selectin levels in non-small cell lung cancer patients,[22] while Setiawan et al found no change in soluble P-selectin values among cancer patients undergoing chemotherapy.[23] Comparable findings were also reported by Haznedaroglu et al and Blann et al.[24,25]

Those with breast and prostate cancer exhibited the highest levels of soluble P-selectin, followed by lung cancer patients. This finding is consistent with research by Cihan Ay et al[21], which showed that high plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients. Additionally, Omunakwe identified higher levels of P-selectin among prostate cancer patients.[26]

In our study, the Khorana risk score (KRS) for most subjects ranged from 1 to 2 (73%), indicating intermediate risk, while approximately 20% were classified as high risk. This suggests that the risk of a thrombotic event within six months is approximately 6.6% for the intermediate-risk group and 11% for the high-risk group, as reported by Mulder et al.[27] Similar findings were noted by Nishimura in a literature review on predicting venous thromboembolism using the Khorana score.[28] Furthermore, a study conducted by Overvad et al[29] in Denmark indicated that cancer patients with a high KRS have a heightened six-month risk of both arterial thrombosis and other thromboembolic events.

Our study also revealed a significant association between KRS and cancer types, which contrasts with the findings of a meta-analysis by Nick Van et al that noted an association of the risk score predominantly with pancreatic cancer.[30] This discrepancy may result from our study being cross-sectional with a broader range of cancers compared to the smaller sample sizes typically used in retrospective studies.

Nevertheless, our findings are similar to those of Keziah et al[31] in a retrospective cohort study of venous thromboembolism rates in ambulatory cancer patients, as well as Ayane Oba Aonuma's research on cancer-associated thromboembolism incidence in Japanese populations.[32] Ellen Marcus also reported a strong association between the Khorana score and venous thromboembolism among ovarian cancer patients.[33]

Lastly, the correlation between P-selectin and the Khorana risk score showed a weak negative association ($r = -0.08$) with a p-value indicating insignificance ($p = 0.404$). This implies no statistically significant association between P-selectin values and the KRS, consistent with the findings of Ay Chan et al.[21]

Conclusion

This study has shown that the risk of VTE among cancer patients ranges from intermediate to high, going by the modified Khorana risk score irrespective of the P selectin level. There is little adherence to Khorana score in our setting, hence the need for greater application and knowledge of this predictive score in clinical practice to improve outcome and quality of life. Furthermore, there is underutilization of thromboprophylaxis among cancer patients and mainly patients at high risk of VTE.

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

No conflict of interest.

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Determine Complete Blood Count Reference Values Among Healthy Adult Populations

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Background: Complete blood counts (CBC) are commonly used in diagnostic medicine to evaluate normal and abnormal hematological status. Furthermore, reference values (RVs) of CBC supplied by researchers are the most reliable means of the judgment-making stage and can aid interpretation and accurate diagnosis of diseases. Reference values vary between peoples because of differences in lifestyle, dietary habits, ethnicity and environment. Moreover, the Clinical and International Standards Institute (CISI) advises determining the RVs for each area. There are no RVs for CBC in Yemen. Therefore, this study aimed to determine the common RVs of CBC for healthy adults in Ibb City in the middle of Yemen.

Methods: A cross-sectional study was conducted from April 1, to November 30, 2023. Of the 623 adults who participated in this study, 433 (aged 18–80 years) were included in the final analysis after applying exclusion criteria. The mean, median, and 95th percentile RVs (2.5th-97.5th percentiles) were calculated for gender, age, and residence by the GraphPad Prism 8.0.1.

Results: The RVs of hemoglobin (Hb) 11.16–17.54g/dl, red blood cells (RBC) 3.890–6.340×10^{12}/l, hematocrit (HCT) 33.03–49.30%), mean corpuscular volume (MCV) 72.83–94.55fl), mean corpuscular hemoglobin (MCH) 23.95–33.55pg, mean corpuscular hemoglobin concentration (MCHC) 32.97–36.7354g/dl, platelet (PLT) count 140.0–418.6×10^{9}/l, total white blood cells (WBC) 2.810–8.797 ×10^{9}/l and WBC differential count (basophils 0.000–1.000%, neutrophils 30.10–69.17%, eosinophils 1.500–5.000%, lymphocytes 23.86–63.45% and monocytes1.873–5.600%). Significantly higher median values were observed in males compared to females for Hb (P<0.0001), RBC (P<0.0001), HCT (P<0.0001), lymphocyte (P=0.0197) and monocytes (P=0.0009). Contrariwise, females demonstrated significantly higher neutrophils (P=0.0009), eosinophils (P=0.0020), basophils (P<0.0001) and platelets (P=0.0324) than males. This study showed differences in the RVs of CBC compared to those reported in other countries in the Middle East, Asia, Africa, and Europe.

Conclusion: In this study, the reference values of CBC are considered as a benchmark that may assist in accurately judging laboratory results and enhancing medical and clinical services for adults in Ibb City, Yemen.

Keywords: reference values, RVs, adults, CBC, Ibb, Yemen

Introduction

Reference values (RVs) are the range of values between and involving lower and upper limits, which were derived from the study of a considerable number of healthy people, to which results can be compared to assist interpretation.[1–4] These typically mean 95% of the results between 2.5th and 97.5th percentiles of the laboratory test values for reference people.[1–3] These usually mean 95% of the results between 2.5th and 97.5th percentiles of the laboratory test values for reference people.[5] Complete blood counts (CBC) are commonly used in diagnostic medicine to evaluate normal and abnormal hematological status.[5,6] Moreover, the RVs of CBC parameters are affected by several factors such as lifestyle, dietary habits, ethnicity, environment, sex, age, pregnancy,[3,7,8] exercise, stress, geography[5,9,10] and environmental elements such as climate and altitude.[11–14] Furthermore, RVs of CBC have not yet been determined in many developing countries, but depend on literature,[15–18] textbook[5,19–21] or brochures of the reagent kits[1,3,22] which rely on specimens obtained from developed countries people.[1,3,5,15–22] Additionally, several studies accomplished in African and Asian countries indicate

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distinctions in RVs compared to those determined in American and European countries.[8,19,20,23–26] Likewise, other studies in the Middle East have informed discrepancies in RVs.[2,15,27–29] Moreover, the Clinical and International Standards Institute (CISI) and the International Federation of Clinical Chemistry (IFCC) have advised establishing of RVs for each area.[3,5,30] Moreover, disparities and variations of RVs in different countries cannot be used for the whole population, and there are no RVs for CBC in Yemen. Therefore, this study aimed to determine CBC RVs among adults in Ibb City, Yemen.

Material and Methods

Study Design, Period and Area

A cross-sectional study was conducted from April 1 to November 30, 2023. The study society is in Ibb City, approximately 193 km south of Sana'a. Ibb City at 13°58′N and 44°10′E with an altitude of 6,725 feet (2,050 meters) above sea level. CBC tests were carried out at the hematology department of Alpha Medical Laboratories.

Sample Size and Sampling Techniques

The subjects of this study were selected from healthy adults (volunteers) aged between 18 and 80 years. According to the Clinical and Laboratory Standards Institute (CLSI) recommendation, a minimum of 120 individuals are enough to determine reference values.[31–33] To maximize the confidence, the investigators increased the number of participants to 623. Of the 623 adults selected randomly from the society of the study, 433 were elected, while 190 (30.50%) were excluded through a medical health questionnaire and physical examination by a physician during the recruitment process.

Exclusion criteria were consumption of any nutritional supplements (vitamins and minerals) or any medication, having a history of diseases (anemia, diabetes, liver disease, heart disease, kidney disease, high blood pressure, arthritis and thyroid disorders), fever, tonsillitis, appendicitis, allergies skin rash or receiving or donating blood within the past year. In case of females who were menstruating, pregnant or breastfeeding were excluded. Furthermore, of the 453 participants who consented to the study through a sampling technique, 20 were excluded based on serological test outcomes (hepatitis C virus (HCV) antibodies, hepatitis B surface antigen (HBsAg) and C-reactive protein (CRP) agglutination test level (≥6 mg/l). Of 433 participants were included in the final analysis after employing exclusion criteria for establishing RVs of CBC parameters for adults (253 males and 180 females) in Ibb City, in the middle of Yemen.

Sampling Methods and Analysis

The participants were interviewed using a standardized questionnaire. Data collected included socio-demographic and medical history information. Approximately 2.5 mL of blood was collected using an EDTA-K3 (Jiangsu Xinkang Medical Instrument Co., Ltd, China). Blood samples were sent to the laboratory and tested immediately for HCV antibodies, HbsAg, CRP and CBC. CBC was measured using a Mindray BC-5000 automatic hematology analyzer (Shenzhen Mindray Bio-Medical Co., Ltd., China). The CBC parameters include hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count and WBC differential count (basophils, neutrophils, eosinophils, lymphocytes and monocytes).

Quality Assurance

The data quality was verified using a standard questionnaire designed in English with consistency and high accuracy and translated into Arabic (the country language). The questionnaire contents were collected from previous research to reduce errors and bias. Further, the research team trained before collecting data and sampling to minimize observer and technological bias. Mindray BC-5000 automatic hematology analyzer (Shenzhen Mindray Bio-Medical Co., Ltd., China) was calibrated according to the manufacturer's guidelines. Mindray BC-5800 controls were conducted before and after testing the samples.

Statistical Analysis

The results of the laboratory tests and questionnaires were entered into a Microsoft Excel spreadsheet. Statistical analyses were achieved using GraphPad Prism 8.0.1 (GraphPad Inc. USA). Ringoringo et al reported flexibility in determining the RVs depending on the desire, either by calculating mean-2SD or determining the lower and upper limits at and between 2.5th-97.5th, 3rd-97th, or 5th-95th percentiles.[34,35] On the other hand, Zeh et al, Fiseha et al and Fondoh et al used the mean, median and RVs percentiles.[29,36,37] Accordingly the mean, median and nonparametric 95% RVs (2.5th and 97.5th percentiles) were calculated for CBC according to CLSI guidelines.[32] The ROUT method (Q = 1%) was used to identify outliers. The normality of the results was determined using the Shapiro–Wilk, Anderson-Darling and Kolmogorov–Smirnov tests. Furthermore, descriptive statistics were used to calculate and compare means, medians and 2.5th-97.5th percentiles between each variable such as gender. The potential disparities in RVs were tested using the Mann–Whitney *U*-test. *P* values ≤0.05 were counted statistically significant.

Results

A total of 433 healthy adults (253 males and 180 females) were included in the analysis to establish the RVs of CBC for adults. As revealed in Table 1, the mean age and standard deviation of the participants were 29.72 ± 10.64 years, ranging from 18 to 80 years and the mean body weight and standard deviation were 58.04 ± 10.04 Kg. The majority of the participants (72.28%) were from urban communities.

Table 2 presents the mean, median and 95% reference values (RVs) (2.5th −97.5th percentiles) of CBC parameters among healthy adults according to gender. The combined mean, median and RVs for both males and females were as follows: 14.33, 14.30 (11.16–17.54g/dl) for Hb, 4.835, 4.800 (3.890–6.340×10^{12}/l) for RBC, 40.90, 40.80 (33.03–-49.30%) HCT, 85.73, 86.20 (72.83–94.55fl) for MCV, 30.06, 30.40 (23.95–33.55pg) for MCH, 35.02, 35.10 (32.97–-36.73 g/dl) for MCHC, 5.245, 5.100 (2.810–8.797×10^{9}/l) for WBC, 49.36, 48.95 (30.10–69.17%) for neutrophils, 43.31, 43.60 (23.86–63.45%), 3.453, 3,500 (1.500–5.0000%) for eosinophil, 3.931, 4.000 (1.873–5.600%) for monocyte, 0.335, 0.000 (0.000–1.000%) for basophil and 270.4, 269.0 (140–418.6×10^{9}/l) for platelets. Significantly higher median values were observed in males compared to females for Hb, RBC, HCT, lymphocytes and monocytes. Contrariwise, females demonstrated significantly higher neutrophils, eosinophils, basophils and platelets than males. Moreover, MCH and MCHC were statistically significant differences.

The combined mean, median and RVs of absolute neutrophils (2.643, 2.465 and 0.990–5.170×10^{9}/l), absolute lymphocytes (2.195, 2.165 and 23.86–63.45×10^{9}/l), absolute eosinophils (0.175, 0.1600 and 0.0460–0.384×10^{9}/l) absolute monocytes (0.217, 0.2000, and 0.077–0.433×10^{9}/l) and absolute basophils (0.017, 0.000 and 0.000–0.070×10^{9}/l). Absolute eosinophils and absolute basophils were statistically significant differences according to gender.

Table 1 Demographic Characteristics of Participants

Characteristics (No.=433)	Mean ± Standard Deviation
Age	29.72±10.64 years
Weight	58.04±10.04 Kg
Sex	No. (%)
Male	253 (58.43%)
Female	180 (41.57%)
Residential areas	No. (%)
Urban	313 (72.29%)
Rural	120 (27.71%)

Table 2 Mean, Median and 95% Reference Values (RVs) (2.5^{th} –97.5^{th} Percentiles) of CBC Parameters Among Healthy Adults According to Gender

Parameters	Gender	Mean	Median	95% CI	RVs	*p*-value
Hb (g/dl)	Combined	14.33	14.30	14.10–14.60	11.16–17.54	<0.0001
	Male	15.31	15.30	15.10–15.60	12.73–17.60	
	Female	13.06	13.00	12.90–13.20	10.65–15.35	
RBC ($\times 10^{12}$/l)	Combined	4.835	4.800	4.690–4.880	3.890–6.340	<0.0001
	Male	5.094	5.100	5.040–5.160	4.138–6.234	
	Female	4.417	4.370	4.300–4.430	3.814–5.458	
HCT (%)	Combined	40.90	40.80	40.30–41.50	33.03–49.30	<0.0001
	Male	43.45	43.60	43.30–44.10	36.54–49.30	
	Female	37.40	37.40	37.00–37.70	31.80–43.44	
MCV (fl)	Combined	85.73	86.20	85.60–86.70	72.83–94.55	0.6049
	Male	85.82	86.30	85.90–86.90	72.48–94.38	
	Female	85.53	86.00	84.70–87.00	72.33–94.81	
MCH (pg)	Combined	30.06	30.40	30.20–30.50	23.95–33.55	0.0413
	Male	30.24	30.40	30.30–30.70	23.85–33.85	
	Female	29.84	30.10	29.70–30.50	24.02–33.50	
MCHC (g/dl)	Combined	35.02	35.10	35.00–35.20	32.97–36.73	<0.0001
	Male	35.18	35.30	35.10–35.40	32.93–36.77	
	Female	34.83	34.90	34.80–35.00	32.94–36.76	
WBC ($\times 10^{9}$/l)	Combined	5.245	5.100	4.890–5.260	2.810–8.797	0.6098
	Male	5.231	4.970	4.700–5.290	2.850–8.944	
	Female	5.262	5.200	4.850–5.370	2.748–8.462	
Neutrophils (%)	Combined	49.36	48.95	47.80–50.00	30.10–69.17	0.0009
	Male	47.93	47.00	45.80–49.30	28.04–66.83	
	Female	51.11	50.60	48.90–51.90	35.68–71.02	
Neutrophils ($\times 10^{9}$/l)	Combined	2.643	2.465	2.532–2.753	0.990–5.170	0.0610
	Male	2.579	2.330	2.425–2.732	0.950–5.461	
	Female	2.728	2.615	2.571–2.884	1.111–4.967	
Lymphocyte (%)	Combined	43.31	43.60	42.50–44.70	23.86–63.45	0.0197
	Male	44.39	45.00	43.00–46.70	24.54–66.23	
	Female	41.98	42.45	40.40–43.60	21.23–58.40	
Lymphocyte ($\times 10^{9}$/l)	Combined	2.195	2.165	2.135–2.254	1.247–3.556	0.1023
	Male	2.236	2.200	2.159–2.314	1.292–3.596	
	Female	2.139	2.105	2.045–2.232	1.070–3.405	

(*Continued*)

Table 2 (Continued).

Parameters	Gender	Mean	Median	95% CI	RVs	*p*-value
Eosinophils (%)	Combined	3.453	3.500	3.500–3.500	1.500–5.000	0.0020
	Male	3.592	3.500	3.500–4.000	1.500–5.000	
	Female	3.283	3.500	3.000–3.500	1.500–5.300	
Eosinophil ($\times10^9$/l)	Combined	0.175	0.1600	0.167–0.1844	0.0460–0.384	<0.0001
	Male	0.190	0.1750	0.178–0.2023	0.050–0.4153	
	Female	0.153	0.1400	0.141–0.1646	0.040–0.3380	
Monocyte (%)	Combined	3.931	4.000	3.800–4.100	1.873–5.600	0.0009
	Male	4.078	4.200	4.000–4.300	1.900–5.600	
	Female	3.751	3.700	3.500–4.000	1.800–5.500	
Monocyte ($\times10^9$/l)	Combined	0.217	0.2000	0.208–0.226	0.077–0.433	0.3686
	Male	0.221	0.2100	0.209–0.2334	0.0755–0.454	
	Female	0.212	0.2000	0.198–0.2264	0.0717–0.428	
Basophils (%)	Combined	0.335	0.000	0.000–0.000	0.000–1.000	<0.0001
	Male	0.000	0.000	0.000–0.000	0.000–0.000	
	Female	0.371	0.000	0.000–0.000	0.000–1.000	
Basophils ($\times10^9$/l)	Combined	0.017	0.000	0.0144–0.019	0.000–0.070	<0.0001
	Male	0.000	0.000	0.000–0.000	0.000–0.000	
	Female	0.018	0.000	0.0147–0.022	0.000–0.070	
PLT ($\times10^9$/l)	Combined	270.4	269.0	260.0–276.0	140.0–418.6	0.0324
	Male	264.1	261.0	247.0–269.0	147.7–411.2	
	Female	278.2	278.5	269.0–291.0	140.3–424.1	

Abbreviations: CI, confidence interval; RVs, reference values; Hb, Hemoglobin; RBC, red blood cells; HCT, hematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; PLT, platelet.

Discussion

RVs provide necessary data to help individual health evaluation.[36] Moreover, RVs are affected by several factors.[38,39] To the best of our knowledge, studies on CBC RVs in Yemen are nonexistent. RVs depend on the manufacturers' guidelines and textbooks, which are often established by non-Yemeni populations. Thus, this study aimed to determine the RVs of CBC in Ibb City, Yemen.

Briefly, the RVs in this study for CBC were varied (more or less) in numbers, decimal numerals, or percentages from the reference values of other studies. The RVs of the CBC parameters may agree or disagree with RVs reported in other studies conducted in Saudi Arabia,[5] Oman,[28] Kuwait,[30] Iraq,[2] Morocco,[3] Ethiopia,[29] Iran,[27] Pakistan,[1] the United Kingdom (UK)[40] and France[41] as shown in Table 3.

In this study, males had higher Hb, RBC, HCT, lymphocytes and monocytes than females. These results are consistent with those reported in previous studies.[1–3,5,27–30,40,41] This may be partly a result of the impact of loss of menstrual blood and androgen hormone on erythropoiesis.[42–44] Moreover, the findings of females have higher levels of neutrophil, which are similar to studies in Saudi Arabia,[5] Iraq,[2] Ethiopia,[29] and France.[41] In addition, eosinophil percentage in females is

Table 3 The Comparison of Reference Values (RVs) Obtained in This Study with Studies in Different Countries

Parameter	Gender	Reference Values (RVs)										
		Our study	Saudi Arabia[5]	Oman[28]	Kuwait[30]	Iraq[2]	Morocco[3]	Ethiopia[29]	Iran[27]	Pakistan[1]	UK[40]	France[41]
Hb (g/dl)	C	11.16–17.54	–	–	–	–	–	11.2–16.8	–	–	–	–
	M	12.73–17.60	12.9–17.9	12.4–16.4	13.8–16.4	15.4–15.6	13–17.1	11.3–17.5	12.3–16.8	12.3–16.6	13.–17	13.4–16.7
	F	10.65–15.35	11.4–15.4	11–15.1	11.3–13.9	13.5–13.7	11–14.8	10.8–16.1	11.2–15.4	11.0–14.5	12.–15	11.8–15.0
RBC ($\times 10^{12}$/l)	C	3.890–6.340	–	–	–	–	–	3.98–6.12	–	–	–	–
	M	4.138–6.234	5.2–5.7	4.45–6.75	4.7–5.8	5.1–5.2	4.37–5.96	3.81–6.38	4.32–6.01	4.25–6.02	3.8–5.5	4.39–5.68
	F	3.814–5.458	4.5–5.0	4.07–6.17	4.1–5.0	4.5–4.6	3.86–5.2	4.06–5.85	4.06–5.62	3.61–5.2	3.6–4.8	3.96–5.12
HCT (%)	C	33.03–49.30	–	–	–	–	–	35.4–52.0	–	–	–	–
	M	36.54–49.30	40–50	36–47	41–50	44.9–45.5	38.3–50	35.2–53.9	35–47	38.4–50.7	40–50	39.2–48.6
	F	31.80–43.44	40–50	33–43	34–42	39.5–40.0	33.5–43.9	35.4–49.8	32–42	34.5–45.4	35–45	34.7–44.4
MCV (fl)	C	72.83–94.55	–	–	–	–	–	77.9–93.8	–	–	83–101	–
	M	72.48–94.38	77.4–94.6	62.5–88.5	80–93	86.2–87.1	77.4–94.2	77.0–93.6	65.3–90.1	78.7–96.3	–	80.20–95
	F	72.33–94.81	76.6–94.2	62.5–88.5	77–92	86.2–87.1	75.1–94.7	78.5–96.4	64.1–89.6	78.1–95.3	–	78.4–95.3
MCH (pg)	C	23.95–33.55	–	–	–	–	–	24.7–32.0	–	–	26–32	–
	M	23.85–33.85	24.5–31.9	20.81–31.2	26–32	29.6–29.9	25.2–32.3	24.7–32.4	22.1–32.9	25.1–31.6	–	27.2–32.8
	F	24.02–33.50	24.8–31.1	20.81–31.2	25–31	29.8–30.1	24–32.3	25.7–32.0	21.7–32.9	25.3–31.7	–	–
MCHC (g/dl)	C	32.97–36.73	–	–	–	–	–	30.6–34.9	–	–	31–35	–
	M	32.93–36.77	30.4–34.5	31–37.2	32.7–34.7	34.2–34.3	31.7–36	30.4–34.9	32.9–37.8	30.0–35.5	–	32.4–36.3
	F	32.94–36.76	30.5–33.9	31–37.2	32.3–34.1	34.2–34.3	31.2–36 3	30.7–34.9	32.8–37.7	30.3–34.4	–	–

WBC (×10^9/l)	C	2.810–8.797	–	–	–	–	–	3.49–11.3	–	–	4–11	–
	M	2.850–8.944	3.7–10.6	2.79–8.09	5.9–12	6.7–7.0	4.1–10.8	3.54–10.9	4.1–9.3	4.88–11.4	–	4.08–10.81
	F	2.748–8.462	3.5–10.6	2.79–8.09	5.4–11	7.1–7.5	4.1–10.7	3.47–11.8	4.1–9.3	4.6–10.8	–	–
Neutrophils (%)	C	30.10–69.17	–	–	–	–		34.95–62.3	–	34.9–76.2	50–72.7	–
	M	28.04–66.83	43.2–55.3	32.6–56.98	46–72	55.2–55.7	43.9–64.8	20.9–59.35	–	–	–	44.71–65.1
	F	35.68–71.02	46.4–61.2	32.6–56.98	49–73	57.7–58.6	43.9–65.4	36.0–66.7	–	–	–	44.61–68.3
Lymphocyte (%)	C	23.86–63.45	–	–	–	–	–	30.6–37.43	–	17.5–45	25–31.8	–
	M	24.54–66.23	32.3–44.2	38.9–40.1	18–41	32.8–34.3	29.3–35.2	30.2–35.2	26.6–55.7	–	–	31.2–36.0
	F	21.23–58.40	29.7–41.7	38.9–40.1	18–40	–	29.3–35.5	25.4–36.0	26.3–55.7	–	–	32.3–35.0
Eosinophils (%)	C	1.500–5.000	–	–	–	–	–	0.6–4.1	–	0.3–7.4	0.25–6.4	–
	M	1.500–5.000	0.4–7.1	1.08–4.57	0.1–6	–	0.0–5.55	0.56–4.4	–	–	–	0.98–5.35
	F	1.500–5.300	0.0–7.0	1.08–4.57	0.1–4	–	0.0–4.67	0.28–3.5	–	–	–	1.02–5.00
Monocyte (%)	C	1.873–5.600	–	–	–	–	–	2.86–8.93	–	3.9–10	2.5–9.1	–
	M	1.900–5.600	5.0–12.4	8.9–8.2	5.6–10	–	4.87–11.1	3.38–9.20	1.7–5.7	–	–	5.65–7.0
	F	1.800–5.500	4.4–12.3	8.9–8.2	4.9–10	–	4.87–11.2	2.30–8.89	–	–	–	5.10–6.35
Basophils (%)	C	0.000–1.000	–	–	–	–	–	0.0–0.79	–	0.10–1.0	0.25–0.9	–
	M	0.000–0.000	0.2–1.3	0.07–0.62	0.2–1	–	0.0–0.74	0.0–0.82	–	–	–	0.0–0.86
	F	0.000–1.000	0.1–1.1	0.07–0.62	0.1–1	–	0.0–0.75	0.0–0.76	–	–	–	0.0–0.76
PLT (×10^9/l)	C	140.0–418.6	–	–	–	–	–	131–391	–	154–433	120–410	–
	M	147.7–411.2	213–283	146–347	184–304	139–339	145–338	130–395	161–337	–	–	166.5–395.5
	F	113.3–424.1	229.2–327.2	164–368	204–350	158–405	150–378	144–434	174–363	–	–	186–432.5

Abbreviations: C, combined; M, male; F, female; g/dl, gram per deciliter; fl, femtoliter; pg, picogram; RVs, reference values; Hb, Hemoglobin; RBC, red blood cells; HCT, hematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin;; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; PLT, platelet.

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higher than that in males, this report is different than studies in Saudi Arabia,[5] Kuwait,[30] Morocco[3] and Ethiopia.[29] Likewise, the basophil percentage in females is higher than that in males, which disagrees with studies done in Saudi Arabia,[5] Kuwait,[30] Ethiopia[29] and France.[41] It be related to geographic dissimilarities, ethnic background, sociodemographic disparities, social lifestyle and habits, dietary customs, ecological factors and laboratory diagnostic techniques.[5,42,45]

Our results showed that the RVs for MCV, MCH and MCHC were almost equal between males and females. These outcomes are consistent with studies accomplished in Saudi Arabia,[5] Oman,[28] Iraq[2] and Pakistan.[1] Additionally, the RV for PLT was higher in females compared to males as reported in Saudi Arabia.[5] It is probably due to menarche through cross-motivating megakaryopoiesis.[42,46] This study's limitation is that the RVs of the CBC parameters in neonates and children are missing.

Conclusion

In conclusion, the RVs of CBC established in the current study are considered a benchmark that may assist in interpreting and accurately judging laboratory results for adults in Ibb City, Yemen. The results displayed significant differences between males and females in Hb, RBC, HCT, MCH, MCHC, neutrophils, lymphocytes, eosinophils, monocytes, basophils and platelets.

Abbreviations

C, combined; M, male; F, female; g/dl, gram per deciliter; fl, femtoliter; pg, picogram; RVs, reference values; Hb, Hemoglobin; RBC, red blood cells; HCT, hematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; PLT, platelet.

Data Sharing Statement

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available because they contain information that can compromise the privacy of the research participants.

Ethical Considerations

The Medical Laboratories Department, Faculty of Medicine and Health Sciences, Ibb University Ethical Committee, granted ethical approval for this research after following due process (Reference No.: MDL-MHS-IBBU/IBA002/2023 dated February 6, 2023). The Research Ethics Committee complied with the Declaration of Helsinki for the Protection of Human Subjects. Participants were informed about the study and provided written informed consent.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, data acquisition, analysis and interpretation, or in all these areas; participated in drafting, revising or critically reviewing the article. Final approval was given for publication; all authors have agreed to the journal to which the article has been submitted and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Open Access Full Text Article CASE REPORT

Deep Vein Thrombosis as a Complication of Gemcitabine-Capecitabine Chemotherapy in Adenocarcinoma of Gallbladder

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Abstract: Gallbladder adenocarcinoma has a high mortality rate, with approximately 1.7% cancer-related deaths worldwide. Cancer-associated thrombosis (CAT), including deep vein thrombosis (DVT), can significantly increase the risk of mortality within cancer patients, especially in pancreatic, brain, and intra-abdominal cancers, as well as in advanced and metastatic cancers. In this case report, there was a 45-year-old male patient diagnosed with advanced gallbladder adenocarcinoma UICC stage IVB with a TNM stage of T2b, N0, M1 with liver metastases who experienced pain and swelling in both lower limbs after undergoing a VI-A cycle of chemotherapy with gemcitabine capecitabine. The risk of thrombosis was calculated using the modified Khorana-Vienna CAT scores, which increased during every chemotherapy session. In this case, the Khorana-Vienna CAT score was calculated during two latest cycle of chemotherapy that somewhat considered delayed as the patient had already shown hypercoagulopathy symptoms and developed a poorer prognosis. Early CAT scoring, ideally before starting chemotherapy session, potentially improves thrombosis prognosis. The patient's condition improved after administration of antithrombotic agents. Chemotherapy agents and other factors, including the cancer site and presence of metastatic cancer, influence the risk of CAT. Risk predictor scores are required to assess the risk of CAT and benefits of prophylactic treatment. Prophylactic therapy can be initiated in patients with high-risk CAT, calculated using the modified Khorana and Vienna CAT scores, to prevent thrombosis and improve patient outcomes.

Keywords: gallbladder cancer, deep vein thrombosis, cancer-associated thrombosis, Khorana-Vienna CAT score, chemotherapy, thromboprophylaxis

Introduction

Gallbladder adenocarcinoma is a highly lethal and rare cancer that emerges in the epithelial layer of the gallbladder.[1] Moreover, the gallbladder is a sac located beneath the hepatic lobe responsible for storing bile fluid produced in the liver, which is then transported to the small intestine. Bile fluid is deposited in the duodenum and contributes positively to digestion, particularly in the lipid metabolism. The accurate incidence of gallbladder cancer is uncertain owing to challenging diagnostics and rapid disease progression, especially in developing countries with limited facilities.[2] Gallbladder adenocarcinoma has a high mortality rate, accounting for approximately 1.7% of cancer-related deaths and a 5-years survival rate of <5% worldwide. Clinically, it often occurs without any symptoms at the early stage of gallbladder adenocarcinoma. The symptoms might only appear in the late stage of the cancer; thus, the prognosis is poor.[2,3] The clinical presentation could differ depending on the stage of the disease at the time of assessment. Intense paroxysmal throbbing pain in the right hypochondriac region that radiates to the right shoulder tip with nausea-vomiting is the most common symptoms of gallbladder cancer. A palpable gallbladder mass and jaundice can be found in later stages of gallbladder cancer. Jaundice may result in a worse clinical presentation of gallbladder cancer because it appears

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following tumor invasion, obstruction of the bile duct due to lymphadenopathy or tumor compression, and liver metastases.[4] Surgical resection of the gallbladder is the main therapy for gallbladder carcinoma, although the recurrence rate after radical resection of gallbladder cancer is still high.[5]

Chemotherapy should be administered as an adjuvant therapy after gallbladder resection surgery to decrease the recurrence rate. The National Comprehensive Cancer Network (NCCN) recommends administering systemic chemotherapy for unresectable or metastatic gallbladder carcinoma.[6] The suggested systemic therapy for biliary tract cancer is currently divided into two categories, one is designated for patients with superior performance status and the other is specialized for patients with poor performance status. Single-agent therapies, such as gemcitabine, capecitabine, and single-agent fluorouracil, are also beneficial for patients with poor performance status, elderly patients, and patients with concurrent medical conditions as symptom control, compared to supportive therapy alone. Combination therapy can be used to treat biliary tract cancer, gemcitabine with 5-fluorouracil (5-FU) or gemcitabine with capecitabine. Patients with a good performance status will establish a good response to combination therapy that can be administered in the form of gemcitabine/platinum-based regimens (cisplatin or oxaliplatin if cisplatin is contraindicated), 5-FU/platinum-based regimens, gemcitabine/capecitabine, or capecitabine/platinum-based regimens. One study recommends a gemcitabine/cisplatin combination as a first-line systemic therapy; however, other studies suggest that other combinations, such as gemcitabine/oxaliplatin and gemcitabine/capecitabine, have similar efficacy to gemcitabine/cisplatin. The gemcitabine/capecitabine regimen can be administered with a capecitabine dose of 1500 mg/m^2 divided into two doses from days 1 to 14, and gemcitabine 1000 mg/m^2 on days 1 and 8 every 3 weeks.[1,6,7] If systemic therapy does not yield significant outcomes, clinical trial medications, like immunotherapy and targeted therapy, may be considered. Palliative chemotherapy and best supportive care may be an option of last resort for advanced or metastatic gallbladder carcinoma that does not respond to treatment and has a poor prognosis.[6] Some guidelines categorize palliative chemotherapy into first-line, second-line, and third-line regimens, which their administration are determined based on performance score (PS). Patients with a PS of 0–1 may receive palliative gemcitabine-cisplatin chemotherapy, while those with a PS ≥2 may be offered gemcitabine monotherapy. Beyond PS, additional factors such as advanced age at diagnosis, lymph node and distant metastases, poor tumor cell differentiation, gallbladder involvement, and albumin and bilirubin levels significantly impact the outcome of palliative chemotherapy in improving patient quality of life and slowing cancer progression.[8] Gemcitabine and capecitabine combination therapy was applied in this case.

Cancer-associated thrombosis (CAT) is more likely to occur since cancer is a predisposing factor for thrombosis. Thromboembolism risk in cancer patients is seven-fold higher compared with the normal population. Chemotherapy increased the possibility of CAT by 5–10%.[9] Deep vein thrombosis (DVT), a manifestation of CAT, significantly increases the risk of mortality among patients with cancer, especially in pancreatic, brain, and intra-abdominal cancers, as well as in advanced and metastatic cancer.[10,11] Administration of thromboprophylaxis can reduce the risk of a venous thromboembolism (VTE) by up to 50% in high-risk patients. Risk predictor scores are required to assess the risk of CAT and the benefits of thromboprophylaxis in cancer patients, especially those who have undergone chemotherapy. Studies have been conducted to develop a predictive risk model for CAT. Several risk score has already examined for validity and compared with other scores for best outcome, such as the Khorana score, Vienna-CAT score, PROTECHT score, and CONKO score. These scores are required to assess the eligibility of potential cancer patient for thromboprophylaxis therapy.[9,12] The National Comprehensive Cancer Network (NCCN) and the American Society of Hematology (ASH) recommend the Khorana score as a valid and reliable CAT predictive score. The Khorana score alone seems to encounter several limitations, thus Khorana score application together with Vienna-CAT score (Table 1) appeared to have more superior outcome by discriminate better between low and high CAT-risk patients.

Case Presentation

A 45-year-old male diagnosed with advanced gallbladder adenocarcinoma UICC stage IVB with a TNM stage of T2b, N0, and M1 with liver metastases experienced throbbing pain and swelling in both lower limbs after undergoing a VI-A cycle of gemcitabine and capecitabine chemotherapy. The pain was exaggerated with movement but improved when his legs were in a straight position. The color of the legs did not change. Both legs felt warm, and the left leg was warmer than the other. There were no complaints of cough, shortness of breath, pain while swallowing, fever, nausea, or

Table 1 The Modified Vienna CAT Score Integrates the Criteria from the Khorana Score and the Addition of Two Biochemical Parameters.[13–15]

Study	Khorana VTE Risk Assessment Score			Point
(Khorana et al, 2021)[14] (Pabinger et al, 2018)[15]	Site of cancer	Very high risk	Stomach, pancreas	2
		High risk	Lung, lymphoma, gynecology, bladder, testicular	1
	Platelet count		≥350x10^9/L	1
	Hemoglobin and/or use of erythropoiesis-stimulating agents		<10 g/dl	1
	Pre-chemotherapy leucocyte count		>11x10^9/L	1
	Body mass index		≥35 kg/m^2	1
Study	**Vienna VTE Risk Assessment Score Addition**			
(Ay et al, 2010)[13]	D-dimer		≥1.44 μg/mL	1
	sP-selectin		≥53.1 mg/mL	1

Note: Based on current study of Khorana et al in 2021 and Pabinger et al in 2018, Khorana score together with Vienna CAT score can predict VTE prognosis better than Khorana score alone. Khorana CAT score classifies cancer patients into three levels of VTE risk stratification: low-risk VTE (Khorana Risk Score (KRS) = 0), moderate-risk VTE (KRS 1–2), and high-risk VTE (KRS ≥ 3).[13–15]

vomiting. Based on previous medical history, the patient complained of intermittent pain in the right upper abdomen that had been experienced for nine months prior to the first admission to the hospital. Abdominal pain increased, especially during meals, accompanied by nausea without vomiting, decreased appetite, and perceived weight loss of approximately two kilograms per month. The abdominal pain was felt to be increasingly heavy and interfered with activities. Abdominal ultrasonography revealed the presence of multiple gallstones. Therefore, gallstone removal and biliary bypass were performed. During the surgery, a lump was observed in the gallbladder. Subsequently, the operator performed a biopsy to obtain a tissue sample for the anatomical pathological examination of the gallbladder. An anatomical pathological examination revealed a malignant gallbladder tumor.

The histological differentiation could provide the extent or size of the tumor and how far the cancer has grown into the wall of the gallbladder that indicated the tumor (T) grade from UICC (Union for International Cancer Control) TNM staging.[16,17] In this case, microscopic histopathologic examination of the gallbladder biopsy sample showed malignant tumor tissue fragments that formed an irregular glandular arrangement lined with anaplastic epithelium, columnar shape, round oval nucleus, pleomorphic, coarse chromatin, prominent nucleoli, and sufficient cytoplasm as shown in Figure 1. The tumor cells growth invaded perimuscular connective tissue on the side of the liver without extension beyond the

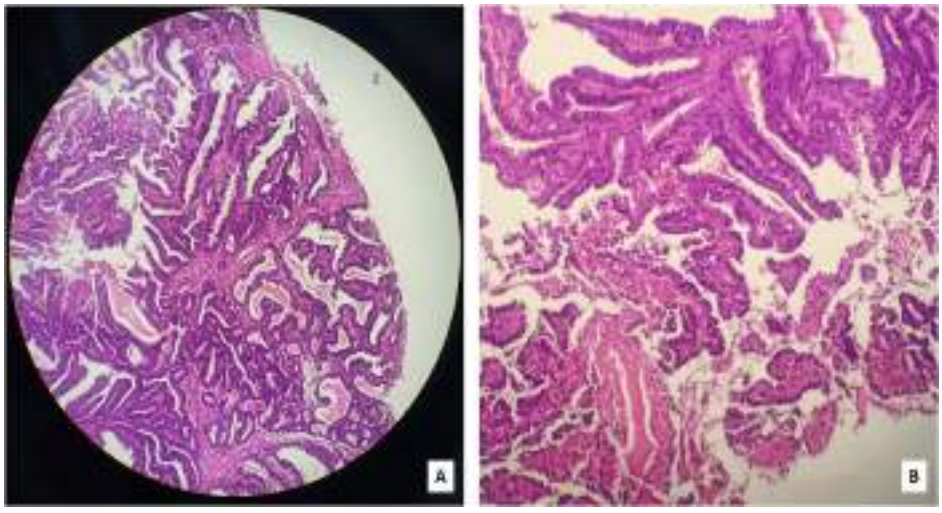

Figure 1 The patient's microscopic histopathologic examination of the gallbladder biopsy sample using total magnification lenses 100x (**A**) and 400x (**B**).

serosa or into the liver (T2b). The conclusion of the gallbladder biopsy material was adenocarcinoma NOS (not otherwise specified). There were no molecular characteristics examinations performed in this case due to the lack of fund and facilities. MRI-MRCP showed a malignant mass in the gallbladder with metastasis to the liver (M), without enlargement or signs of metastasis to the lymph nodes (N). Based on histopathology and radiology examination results, the patient had suffered from gallbladder adenocarcinoma UICC stage IVB with a TNM stage of T2b, N0, and M1 liver metastasis as guided by the UICC staging system.[16,17] Biliary tract cancer, including gallbladder carcinoma and cholangiocarcinoma, has been known to be associated with genetic predisposition that can be detected through molecular testing from germline mutation. The most abnormality findings were BRCA2 mutation followed with BRCA1 mutation, and at least MLH1, MSH2, PALB2, RAD51D, BAP1 and ATM mutation. Recommended molecular testing for gallbladder carcinoma includes ERBB2, ERBB3, ELF3, BRAF V600E mutation, NTRK gene fusion, MSI-H/dMMR, RET gene fusion, and also HER2 mutation.[18,19] In this case, we did not perform molecular testing due to lack of facilities and cost-related issues.

The patient was then advised to undergo chemotherapy that was planned for a total of six cycles every 28 days (with divided doses every 14 days) using a gemcitabine-capecitabine chemotherapy regimen. After the first chemotherapy session (I-A cycle), the patient experienced side effects, such as nausea, vomiting, intermittent right abdominal pain, occasional back pain and right-sided chest pain. After the VI-A cycle of chemotherapy, the patient experienced pain and swelling in both the legs. This patient was diagnosed with hypertension 5 years prior with routine treatment of once daily 10 mg of amlodipine and once daily 20 mg of atorvastatin every night, previous liver disease or jaundice was denied, history of tuberculosis was denied, history of diabetes mellitus was denied, history of kidney disease was denied. There was no history of malignancy with other family members. This patient was a non-smoker, and alcohol consumption was excluded. The patient underwent the I-A cycle until VI-B chemotherapy with 1200 mg of gemcitabine was consumed on day-1 and day-8, accompanied by 1000 mg capecitabine twice daily for seven days.

On physical examination after the VI-A cycle of chemotherapy, hemodynamics were stable: blood pressure was 128/73 mmHg, heart rate was 89 beats per minute, respiratory rate was 18 breaths/min, temperature was 36.8 °C, oxygen saturation was 97% on room air, and oxygen saturation of the lower extremities was 97–98% on room air. A physical examination revealed a distended abdomen, visible collateral veins, liver enlargement (three fingers below the costae arc), and tenderness in the right upper quadrant. The patient showed a positive Homan's sign in both lower limbs. Laboratory examinations were performed after VI-A cycle of chemotherapy to determine the patient's condition. The following values were obtained: hemoglobin, 12.2 g/dl; hematocrit, 37.4%; MCV, 101.6 fl; MCH, 33.2 pg; MCHC, 32.6 g/dl; leukocytes 8200/µL with a dominant neutrophil count of 71.9%; lymphocytes, 15.6%; monocytes, 11.2%; eosinophils, 0.9%; basophils, 0.4%; platelets, 269,000/µL; SGOT, 248 U/L; SGPT, 97 U/L; non-reactive HBsAg; non-reactive Anti-HCV. In this case, signs of hypercoagulopathy were examined using D-dimer serum levels and Doppler ultrasonography examination of lower extremities to evaluate thrombus development, while the predictive risk score for VTE was determined using the modified Khorana-Vienna CAT scores. The Khorana-Vienna CAT score in this case was calculated twice: during VI-A chemotherapy cycle and 14 days after VI-B chemotherapy cycle. These calculations were considered pretty late, because the patient has already developed hypercoagulopathy symptoms and a poorer prognosis. The progression of D-dimer and predictive risk score of DVT increased. During VI-A chemotherapy cycle, D-dimer level was 0.49 ug/mL with a modified CAT score 9.2% and the Khorana-Vienna CAT score 2 or intermediate score. Meanwhile, in 14 days after VI-B chemotherapy cycle, D-dimer level increased to 2.4 ug/mL with progression of modified CAT score to 12.7% and the Khorana-Vienna CAT score 3 or high-risk score (the risk score explanation can be seen in Table 1).

Doppler ultrasound examination of the patient's lower limb vessels revealed evidence of DVT (left: common femoral and popliteal veins; right: common femoral vein). The occurrence of DVT in this patient was suspected to be a complication of gemcitabine because the symptoms of DVT presented before the last cycle of chemotherapy, although disease progression itself might exaggerate the probability of CAT. The patient was administered a subcutaneous injection of fondaparinux sodium 2.5 mg once daily, followed by a subcutaneous injection of 40 mg of enoxaparin sodium once daily for one week (VTE prophylaxis therapy can be seen in Table 2). The patient's condition improved. Swelling and pain in both legs decreased. The patient was advised to continue treatment as an outpatient. Rivaroxaban 10 mg once daily was administered as an ambulatory therapy. After the last cycle of chemotherapy, the patient underwent an MRI chemotherapy response examination which

Table 2 NCCN Cancer-Associated Venous Thromboembolic Disease Guideline Recommendation for Venous Thromboembolism (VTE) Prophylaxis Options.[32]

	Condition	Drug of Choices	Standard Dose	Renal Dose and Other Condition
Prophylaxis for ambulatory medical oncology patient (Streiff et al, 2021)[32]	High risk thrombosis	Apixaban[32]	2.5 mg PO twice daily	Avoid if CrCl<30 mL/min, avoid if platelet<50,000/ul
		Rivaroxaban[32]	10 mg PO once daily	Avoid if CrCl<15 mL/min, avoid if platelet<50,000/ul
		Dalteparin[32]	200 Unit/kg SC daily (1 month) continue with 150 Unit/kg SC daily (2 months)	Avoid if CrCl<30 mL/min, avoid if platelet<50,000/ul
		Enoxaparin[32]	1 mg/kg SC qd (3 months) continue with 40 mg SC once daily	Avoid if CrCl<30 mL/min, avoid if platelet<50,000/ul
Prophylaxis for hospitalized medical oncology patient (Streiff et al, 2021)[32]	Critical, long immobilization, high risk	Dalteparin[32]	5000 Unit SC daily	Avoid if CrCl<30 mL/min
		Enoxaparin[32]	40 mg SC daily	Adjust dose to 30 mg SC daily if CrCl<30 mL/min
		Fondaparinux[32]	2.5 mg SC (weight ≥50 kgs)	Caution if CrCl 30–49 mL/min, Avoid if CrCl<30 mL/min
		UFH[32]	5,000 Unit SC every 8–12 hours	Same as standard dose

Note: Streiff et al in 2021 contributed to establish recommendation for VTE prophylaxis that has been recommended by NCCN Cancer-Associated Venous Thromboembolic Disease Guideline. The administration of anticoagulant can be initiated in patients with a Khorana score of ≥2[14,32].
Abbreviations: VTE, Venous thromboembolism; UFH, unfractionated heparin; PO, per oral; SC, subcutaneous injection; CrCl, estimated creatinine clearance.

showed that the malignant mass of the gallbladder was still prominent, liver metastasis still existed, and hepatomegaly. Because the latest abdominal MRI results showed that the mass in the gallbladder was still present, the patient was then planned for palliative chemotherapy using Gemcitabine monotherapy.

Discussion

Adenocarcinoma of the gallbladder is one of the rarest cancers of the abdominal organ. It has a poor prognosis due to its highly malignant character and is often discovered incidentally during laparoscopic cholecystectomy with a delayed presentation.[20] The International Agency for Research on Cancer (IARC) Globocan in 2018 estimated that gallbladder cancer accounts for 1.7% of cancer-related deaths worldwide, with 220,000 new cases diagnosed each year and a female predominance 3–6 times higher than in males. The highest incidence rates per 100,000 persons were found in Latin America, East Asia, and Eastern Europe, with the highest incidence recorded in Chile (27), followed by Northern India (21.5), Poland (14), Pakistan (11.3), Japan (7), and Israel (5). Incidence in the United States varies significantly by ethnicity, with Native Americans showing a rate of 3.3 compared to non-Native Americans (0.4–1.5), where the incidence is higher in Caucasians than in Black Americans but lower than in Hispanics.[3] More than two-thirds of people diagnosed with gallbladder cancer are over the age of 65, with an average age of 72 years.[2] The early symptoms of gallbladder adenocarcinomas vary. Symptoms experienced by patients manifest as abdominal pain, nausea-vomiting, jaundice, weight loss, and abdominal masses that can be found in physical examination or radiologic imaging.[1] In this case, the patient complained of intermittent upper right abdominal pain, nausea, and weight loss, and cancer was incidentally found during gallstone surgery. Surgery is the gold standard curative therapy for gallbladder adenocarcinoma, with a 5-year survival rate of 63.2%; however, only 10% of patients with early-stage disease are eligible for surgery.[7] The overall median survival rate for unresectable or metastatic biliary tract cancer is less than one year, with gallbladder malignancy becoming the most progressive biliary tract cancer with less than 5% of a 5-year survival rate.[7]

The recurrence rate of post-resection cancer is still high, therefore systemic chemotherapy is still recommended following surgery, either as adjuvant or palliative treatments.[21]

Patients with malignancies are prone to either arterial or venous thrombosis, also known as cancer-associated thrombosis (CAT).[22] The predominant presentation of CAT typically involves VTE, including DVT, which occurs in 5–10% of patients after a year of chemotherapy.[9,12] The increased risk of VTE is influenced by various factors, including the type and stage of cancer, patient characteristics, and the chemotherapy protocol. Patients with pancreatic, brain, and digestive system cancers, advanced-stage cancer, and metastatic cancer have a tendency to develop VTEs. Patients diagnosed with breast, prostate, and early-stage cancers have a lower risk of developing VTE. Meanwhile, patients with brain, lung, uterine, bladder, pancreatic, gastric, and kidney cancers have an incidence of VTE in a year after diagnosis. Furthermore, the risk of VTE increases significantly in patients with metastasis, ranging from 4 to 13 times higher.[10,11] Ultrasonography and contrast-enhanced computed tomography (CT) are recommended for early performance after chemotherapy initiation. Asymptomatic VTE is more threatening because it is more difficult to detect; thus, biomarker examinations should be performed following radiological imaging. Patients with VTE often exhibit increased D-dimer levels, degradation of fibrin products, and interleukin (IL)-6 levels, all of which can be used as potential VTE biomarkers. Monitoring D-dimer levels during and after chemotherapy initiation could serve as a sensitive and straightforward approach for promptly detecting asymptomatic VTE and prevent potential complications.[23] The patient in this case was reported with an increased D-dimer serum level from 0.49 ug/mL after the VI-A-cycle of chemotherapy with 2.4 ug/mL at 14 days after the VI-B-cycle of chemotherapy, whilst the VTE was confirmed by Doppler ultrasound examination of lower extremity that supported the image of DVT by the discovery of thrombus from the level of the common femoral vein and popliteal vein of the left lower extremity, and up to the level of the common femoral vein of the right lower extremity (Figure 2).

The role of gemcitabine in activating the coagulation and hemostasis cascade is still poorly understood. Studies on cancer-associated thrombosis (CAT) in gallbladder carcinoma explained one possible mechanism of that cancer complication involving a protein called sciellin (SCEL), which is highly expressed in epithelial tissues and played important role in stress resilience and barrier functions. SCEL overexpression is highly associated with various cancer progression,

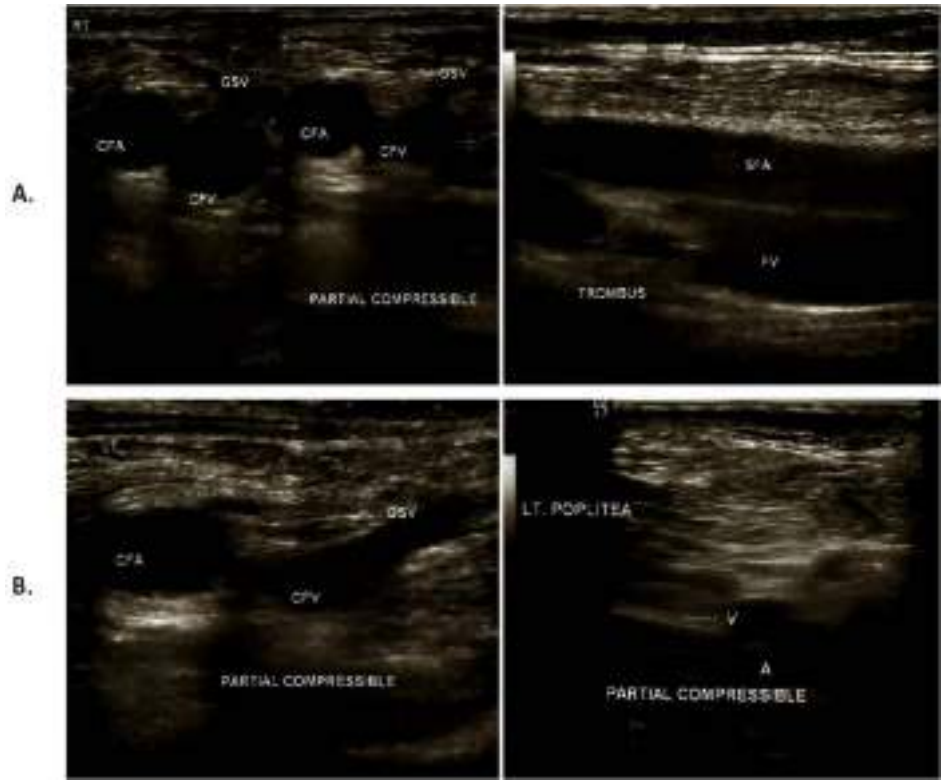

Figure 2 Doppler ultrasound examination of the patient supported the Deep Vein Thrombosis (DVT) image. Thrombus was found from the level of the common femoral vein of the right lower extremity (**A**) and from the common femoral vein and popliteal vein of the left lower extremity (**B**).

including gallbladder carcinoma. SCEL induces interleukin (IL) 8 secretion that lead to the formation of neutrophil extracellular traps (NETs) which attract platelets and contribute to thrombus formation. NET formation is driven by neutrophil releasing chromatin and granular proteins, forming a fibrillar matrix that leads to extensive clotting in the affected area. An animal study found that SCEL-overexpressed group showed higher IL-8 level, increase myeloperoxidase DNA or MPO-DNA (a NETs marker), and larger clot formations compared to normal subjects. Tumour growth factor, tumour-educated platelet, and cytokines, such as granulocyte colony-stimulating factor (G-CSF) and IL-8, increased NETs formation in cancer and worsened progression of the cancer by developing CAT.[24] However, both thrombocytopenia and thrombocytosis are well-known side effects of gemcitabine, one of which may have an important role in thrombophilia-related regulation and balance. Gemcitabine-induced thrombocytopenia follows when this agent causes vascular endothelial changes.[25] Other studies have shown that administering gemcitabine was not associated with a significant increase in VTE. The combined data showed that gemcitabine has a tendency to enhance the risk of VTE in cancer patients, although the exact mechanism is still unclear. Statistically, gemcitabine chemotherapy did not increase the incidence of VTE in cancer patients compared to other chemotherapy regimens.[26] Gemcitabine may cause microvascular thrombosis through the development of thrombotic microangiopathies (TMA). Gemcitabine-related TMA has been previously reported; however, these cases are very rare.[10,27] Previous research using univariate analysis confirmed the relative increased risk of VTE with gemcitabine and platinum-based therapies, both regimens apparently increased vascular toxicity. In the latest multivariate studies, VTE risk was adjusted with the CAT predictive score that categorized the tumor site and D-dimer level between gemcitabine therapy and platinum-based therapy. These findings suggest that most cases of VTE in cancer patients treated with gemcitabine or platinum-based therapy may be related to the underlying thrombotic risk (such as the cancer site) rather than the chemotherapy agent itself.[28]

Table 1 shows that the VTE predictive score recommended by NCCN includes the modified Vienna CAT score and the Khorana score with two additional biomarker parameters from the Vienna CAT score. The National Comprehensive Cancer Network (NCCN) and the American Society of Hematology (ASH) recommend the Khorana score,[29] that developed by Khorana et al in 2008, as a tool to predict and identify VTE risk in patients with cancer. As time goes by, the Khorana score was discovered to have some limitations. The Khorana score itself cannot be applied to all types of malignancies, particularly lymphoid malignancies, since leukocytosis criteria cannot serve as benchmark. In lung and pancreatic cancers, the Khorana score does not accurately represent the risk of VTE, as these malignancies have a huge incidence rate of VTE. Additionally, the Khorana score itself is considered less effective in predicting VTE events in low and moderate risk groups with a KRS of 0–1.[30] The modified Vienna CAT score that was created by Ay et al in 2010 is based on the Khorana VTE Risk Assessment Score (that consists of site of cancer, platelet count, hemoglobin and/or use of erythropoiesis-stimulating agents, leucocyte count before administering chemotherapy, and body mass index) by adding two VTE biomarker parameters, D-dimer and soluble P-selectin.[13] Based on the current study of Khorana et al in 2021 and Pabinger et al in 2018, Khorana score together with Vienna CAT score can depict hemostatic system activation better and can be independent prognostic factors for VTE in cancer (Table 1).[14,15] Based on the current study of Khorana et al in 2021 and Pabinger et al in 2018, Khorana score together with Vienna CAT score can predict VTE prognosis better than Khorana score alone.[14,15] Prognosis of the thrombosis might be improved if the CAT score was performed earlier and advised to be performed before the chemotherapy session started. The risk of CAT increases following chemotherapy administration, thus the Khorana-Vienna CAT score should ideally be re-evaluated before each chemotherapy cycle begins.[31]

The cancer site was the most influential substance in this predictive tool. Modified predictor scores, such as the Vienna CAT modified scores, were established to support the limitations of the Khorana score. The specificity and sensitivity of the scoring system were considered to be low when a single biomarker was used. The specificity of the Khorana scoring system increases with modification of the combination of clinical symptoms and dual biomarkers.[30] Khorana CAT score classifies cancer patients into three levels of VTE risk stratification: low-risk VTE (Khorana Risk Score (KRS) = 0), moderate-risk VTE (KRS 1–2), and high-risk VTE (KRS $\geq$ 3).[14,15]

Table 2 depicts the drug of choices for VTE prophylaxis. Streiff et al in 2021 contributed to establish recommendation for VTE prophylaxis that has been recommended by the NCCN Cancer-Associated Venous Thromboembolic Disease Guideline.[32] This guideline is divided into two groups: prophylaxis for ambulatory patient and for hospitalized patient. In ambulatory setting with high risk thrombosis patient, the drug of choices are apixaban, rivaroxaban, dalteparin, or

enoxaparin. Whilst in a hospitalized setting with critically ill, long immobilization and high-risk thrombosis could be given with Dalteparin, Enoxaparin, Fondaparinux, or UFH (Table 2).[32] The administration of anticoagulant therapy can also be assessed using the Khorana score, with guidelines stating that anticoagulant therapy can be initiated in patients with a Khorana score of ≥2.[9,30] The higher the risk score, the more beneficial is the thromboprophylaxis therapy. Prophylactic therapy can be initiated to prevent thrombosis in patients with high-risk CAT to prevent thrombosis.[11,28,33] Increased CAT predictive scores necessitated the administration of thromboprophylactic therapy in this patient. The patient received a subcutaneous injection of fondaparinux sodium followed by a subcutaneous injection of enoxaparin sodium for seven days, followed by oral administration of rivaroxaban for ambulatory therapy.

Conclusion

A 45-year-old male with advanced gallbladder adenocarcinoma UICC stage IVB with a TNM stage of T2b, N0, M1 with liver metastases and DVT complications after a VI-A chemotherapy cycle was reported. He had a high risk of cancer-associated thrombosis (CAT) based on the calculation of the Khorana-Vienna CAT scores and was administered thromboprophylactic therapy to improve the patient's condition. Early evaluation of CAT predictive score will prevent thrombosis event in cancer patient. Ideally, the Khorana-Vienna CAT score should be evaluated before every cycle of chemotherapy regardless of the regiments to improve patient's prognosis. Chemotherapy agents alone cannot increase the risk of CAT; other factors, including the cancer site and presence of metastatic cancer, also influence the risk of CAT. Some regiments, like Gemcitabine, have a tendency to induce venous thromboembolism, but the mechanism is still unclear. Clinicians can use predictor risk scoring, such as the Khorana-Vienna score, to calculate the risk of CAT and decide whether to initiate thromboprophylaxis in cancer patients before chemotherapy, which can aid in improving patient outcomes.

Ethics and Consent

Written informed consent was obtained from the patient during admission to unveil the case details, including the examination results and other accompanying images, for publication and educational purposes. There was no institutional approval that required for publication.

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Disclosure

The authors report no conflicts of interest in this work.

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Assessment of Knowledge and Medication Adherence Among Patients Prescribed with Oral Anticoagulants in Atrial Fibrillation at a Tertiary Care Centre: A Cross Sectional Study

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Assessment of Knowledge and Medication Adherence Among Patients Prescribed with Oral Anticoagulants in Atrial Fibrillation at a Tertiary Care Centre: A Cross Sectional Study

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Purpose: Atrial fibrillation (AF) being a prevalent cardiovascular condition globally, has an increased risk of stroke and other complications. The effective management of AF often involves the use of oral anticoagulants (OACs) to prevent thromboembolic events. This study aimed to evaluate anticoagulation knowledge and medication adherence in AF patients on OACs at a tertiary care center in Nepal.

Patients and Methods: A descriptive cross-sectional study involving patients diagnosed with AF who were prescribed OACs at the Cardiology Department of Dhulikhel Hospital, Nepal, was conducted from March to June 2024. Data were collected using questionnaires, including the Oral Anticoagulation Knowledge Tool (AKT) and the Adherence in Chronic Diseases Scale (ACDS). The study included patients from the Dhulikhel Atrial Fibrillation (DAF) Registry database along with other AF patients visiting the cardiac department. Descriptive statistics were used to summarize patient demographics, knowledge scores, and adherence levels. Inferential statistics were used to observe the associations.

Results: Among the 114 AF patients enrolled in the study, 93 were receiving OAC therapy and were interviewed. The mean age of the participants was 66.84 ± 12.3 years, with the majority being female (57%). The study revealed that a significant portion of patients lacked adequate knowledge about their OAC therapy, with only 48% having adequate knowledge as per the AKT. Additionally, 83.9% of the patients demonstrated high adherence to their medication regimen, whereas 16.1% showed medium adherence. The duration of use of OACs was found to be significantly associated with adequate anticoagulation knowledge.

Conclusion: The study findings indicate that a significant proportion of AF patients in Nepal lack adequate anticoagulation knowledge, highlighting an opportunity for improved educational interventions.

Keywords: anticoagulation knowledge, knowledge gaps, adherence, stroke prevention

Introduction

Atrial fibrillation (AF), the most prevalent cardiac arrhythmia, occurs when abnormal electrical activity in the atria causes rapid and irregular heartbeats.[1] It is the most common sustained arrhythmia requiring hospital admission.[2] AF has a significant effect on public health, including decreased quality of life, increased hospitalization rates, stroke incidence, and increased medical costs.[3] It has been projected that 6 to 12 million individuals globally are expected to experience this ailment in the United States by the year 2050, with an estimated 17.9 million cases in Europe by 2060.[4,5] The currently estimated prevalence of AF in adults is between 2% and 4%, and a 2.3-fold increase is expected owing to extended longevity in the general population and an intensified search for undiagnosed AF.[6] Australia, Europe, and the USA have the highest reported prevalence of AF (1% in the adult population), but the prevalence of AF in low-income and middle-income countries is probably underestimated.[7] In 2010, there were an estimated 33.5 million cases of AF globally (20.9 million men and 12.6 million women), with significant regional variations and heterogeneity and

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approximately 5 million new cases annually.[8] The overall prevalence of AF in Asia is less than 1%.[9] The prevalence of AF in Nepal was 13.8%, which was higher than that in Western countries, mainly because of endemic rheumatic heart disease.[10] Another study done in Sindhupalchowk, Nepal found AF prevalence to be 11% among the elderly.[11]

Patients with AF in low- or middle-income countries (LMICs) have higher morbidity and mortality rates than do those with AF in high-income countries, with limited data on the economic burden in LMICs. Various studies from different countries, such as Algeria, China, Brazil, and India, have highlighted the economic burden of AF, with costs per patient-year ranging from 1003 USD to 8020 USD.[12]

Oral anticoagulation (OAC) is the most effective treatment for preventing ischemic stroke and systemic embolism associated with AF.[13] Vitamin K antagonists (VKA: Warfarin, Acenocoumarole, Phenindione) and NOAC (Dabigatran, Rivaroxaban, Apixaban) comes under OAC group.[14] NOACs provide advantages over traditional vitamin K antagonists such as warfarin, including reduced bleeding risk, the necessity for less monitoring, and more predictable pharmacological effects.[15] Patients' understanding of their medication and medical condition can influence the effectiveness of their treatment.[16] This becomes especially crucial for patients on oral anticoagulants, given the narrow therapeutic range of these medications and the severe consequences that can result from either undertreatment or excessive anticoagulation.[17] This is why information with respect to medicine is imperative for patients. Better drug-taking practices are influenced by knowledge of the disease, an understanding of why the drug is needed, and positive expectations or attitudes toward the course of therapy.[18] Likewise, adherence to medication is vital for successful treatment, as noncompliance can worsen health conditions, increase healthcare costs, and even lead to fatal outcomes.[19]

There have been few studies on AF, including those specializing in warfarin therapy. A cross-sectional study performed at Shahid Gangalal National Heart Centre (SGNHC) in Kathmandu reported moderate knowledge of anticoagulation and good compliance with medication.[20] Similarly, another study conducted at Manmohan Cardiothoracic Vascular and Transplant Center, Institute of Medicine, reported insufficient use of anticoagulants for both valvular and nonvalvular atrial fibrillation, which may be attributed to economic limitations and geographical challenges.[21] Another study concluded that despite the increasing use of NOACs in patients with a higher risk of stroke, anticoagulants are still underutilized in most cases.[22] In addition to some studies that focused on the prevalence and utilization of AF, knowledge and adherence have not been assessed in AF patients prescribed OACs. Therefore, this study aimed to assess knowledge and adherence among AF patients.

Materials and Methods

Recruitment

This quantitative, cross-sectional study was conducted in the cardiac outpatient department of Dhulikhel Hospital, Nepal, for four months (March to June 2024). The study adhered to the principles outlined in the Declaration of Helsinki and ethical approval was obtained from the Institutional Review Committee of Kathmandu University School of Medical Sciences (IRC-KUSMS Approval Number: 73/24). Patients from the Dhulikhel AF registry and other patients visiting the cardiac outpatient department were included. Patients who had taken any OAC drugs for at least 1 month and had a diagnosis of AF were included in the study through purposive convenience sampling. Written informed consent was obtained from the patients, clearly outlining the study's purpose and its potential impact, while ensuring the confidentiality of all patient information. The study included 114 patients diagnosed with AF. Among those, 93 patients were on OACs and were included in the study and interviewed for AKT and ACDS questionnaires. Patients who were not on OACs or had valvular AF were excluded from the final analysis, leading 83 patients to be analyzed for predictors of NOACs use (Figure 1).

Data Collection Tools

The data collection tool was divided into three parts. The first section focused on the patients' sociodemographic information and clinical characteristics. The second section is the questionnaire on the AKT, and the third section is the ACDS. The participants were asked if they currently had a diagnosis of AF and then asked whether they were on OACs or not and how long they had been taking it. Face–to-face interviews were conducted with patients via the Oral Anticoagulation Knowledge Tool (AKT) and Chronic Disease Adherence Scale (ACDS), which were shown to have

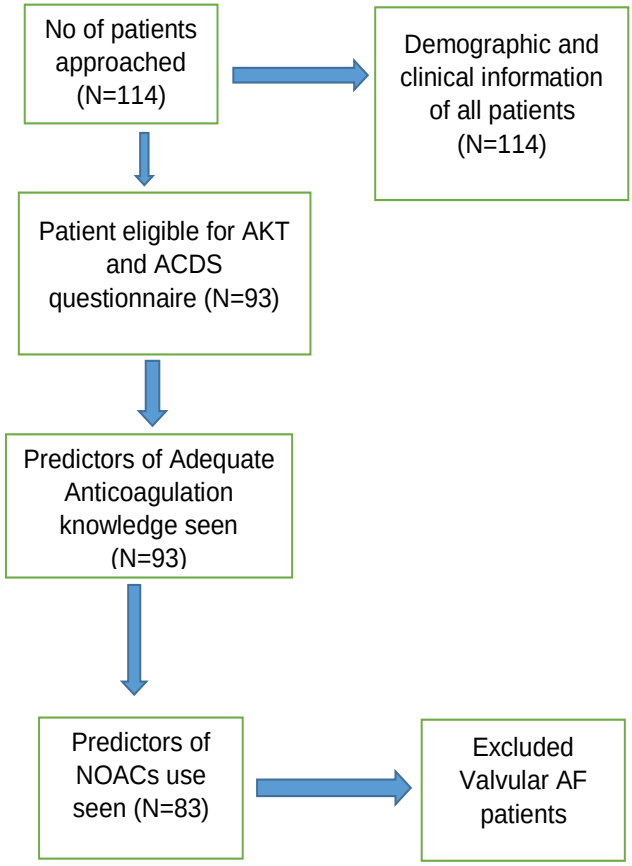

Figure 1 Flowchart of Study Design.
Abbreviations: AKT, Oral Anticoagulation Knowledge Tool; ACDS, Chronic Disease Adherence Scale; NOACs, Non-Vitamin-K Oral Anticoagulants; AF, Atrial Fibrillation.

acceptable validity and reliability in a previous study.[23,24] The questionnaire was translated into the Nepali language and then back-translated to English to retain the previous concept of the questionnaire. The pilot study was performed among 15 patients at Dhulikhel Hospital, and the data were excluded from the final analysis. The reliability of the questionnaire was checked and found to be 0.780.

Oral Anticoagulation Knowledge Tool (AKT)

Anticoagulation knowledge was assessed via the Oral Anticoagulation Knowledge tool, which was developed and validated in patients taking either VKA (Warfarin) or NOACs.[23] The AKT has two sections: general questions (Section A – 20 items) and warfarin-specific questions (Section B – 8 items). All participants were required to answer section A, but only those on warfarin needed to answer section B. Participants on NOACs could achieve a maximum score of 25 points, as they were only required to complete Section A. Those on warfarin could score up to 35 points, needing to complete both Sections A and B.[23] Final scores were presented as a percentage of correct answers for all participants.

Adherence in Chronic Disease Scale (ACDS)

The ACDS is a 7-item questionnaire.[24] The scale for chronic diseases comprises 7 questions, each offering 5 possible answer options. The first 5 questions focus on behaviors that directly influence adherence, whereas the last 2 questions address circumstances and perspectives that might indirectly impact adherence.[24] Scores were calculated as stated in the original reports.[24]

Variables

Dependent Variables: Knowledge, Adherence

Independent variables: Age, gender, level of education, duration since OAC use, length of time since AF diagnosis, CHA_2DS_2VASC score, HASBLED score, smoking, alcohol

Data Analysis

SPSS version 20 was used to analyze the data. Continuous variables are expressed as the means ± SDs. Categorical variables are presented as percentages and definite values. Categorical variables were compared via the chi-square test and Fisher's exact test. A p value less than 0.05 was considered statistically significant. Knowledge of OAC was assessed via total AKT scores, which were determined by counting the number of correct responses and converting them into percentages. A cutoff of > 50% was considered an adequate knowledge score. An adequate knowledge threshold was set at 50% or higher. Thus, participants achieving 50% or more on their respective total score (converted to a percentage) were considered to have adequate anticoagulation knowledge, regardless of whether they were on NOACs or warfarin. Medication adherence scores were calculated by summing the numbers assigned to each response. The overall mean and median scores were calculated for both sections, and the mean scores of both sections were also reported for all demographic groups. The Mann–Whitney *U*-test (for two groups) and the Kruskal–Wallis test (for more than two groups) were used to compare mean scores across different demographic characteristics. A p value of less than 0.05 was considered statistically significant. Binary logistic regression was conducted to identify the factors associated with adequate knowledge scores and predictors of NOACs. Both univariate and multivariate logistic regression analyses were performed. A p value of less than 0.05 was considered statistically significant.

Results

Table 1 presents the baseline characteristics of 114 AF patients. The majority of patients were aged between 18 and 64 years. The mean age was 66.84 years, with a standard deviation (SD) of 12.3. The majority of patients were aged 18–64 years (n=43, 37.7%), followed by those aged 65–74 years (n=38, 33.3%) and 75 years and above (n=33, 28.9%). More than half (57%) of the patients were females. In terms of social habits, the majority of patients were former smokers (67.5%) or former drinkers (56.1%). More than half (85.1%) of the patients were NVAF in origin.

The most common comorbidity was hypertension (n=45, 39.5%), followed by congestive heart failure (n=35, 30.7%), COPD (n= 29, 25.4%), RHD (n=26, 22.8%), and DM (n=16. 14%), hypothyroidism (n=14, 12.3%), stroke (n=12, 10.5%), and CAD (n=6, 5.3%).

The mean CHA_2DS_2VASC score was 2.58 (±1.42). Most of the patients had a CHA_2DS_2VASC score of 3 (n=31, 27.2%), followed by 2 (n=25, 21.9%), 1 (n=24, 21.1%), 5 (n=14, 12.3%), 4 (n=13, 11.4%) and 0 (n=6, 5.3%). The mean

Table 1 Baseline Characteristics of AF Patients (N=114)

Parameter	Overall Sample (n=114)	
	Frequency (n)	Percentage (%)
Gender		
Female	65	57
Male	49	43
Age group of patients		
Age, years, Mean (SD)	66.84 (12.3)	
18 to 64	43	37.7
65 to 74	38	33.3
≥75	33	28.9
Highest education level		
No formal Education	85	74.6
Below SLC	20	17.5
SLC and above	9	7.9

(*Continued*)

Table 1 (Continued).

Parameter	Overall Sample (n=114)	
	Frequency (n)	Percentage (%)
Duration since AF diagnosis		
Less than 2 years	46	40.4
More than 2 years	68	59.6
Employment status		
Employed	7	6.1
Unemployed	107	93.9
Smoking		
Yes	3	2.6
No	34	29.8
Ex-smoker	77	67.5
Alcohol		
Yes	2	1.8
No	48	42.1
Exdrinker	64	56.1
CHA_2DS_2VASC RISK		
CHA_2 DS_2 VASC score Mean(SD)	2.58 (1.42)	
Low risk (0)	6	5.3
Intermediate risk (1)	24	21.1
High risk (≥2)	84	73.7
HASBLED RISK		
HASBLED score Mean (SD)	0.96 (0.775)	
Low risk (0)	34	29.8
Moderate risk (1–2)	78	68.4
High risk (≥3)	2	1.8
BMI of patients		
Severely underweight (< 16.5 kg/m^2)	3	2.6
Underweight (16.5–18.5 kg/m^2)	6	5.3
Normal weight (18.5 to 24.9 kg/m^2)	58	50.9
Obesity (≥30 kg/m^2)	47	41.2
AF Type		
Valvular AF	17	14.9
Non Valvular AF	97	85.1

(*Continued*)

Table 1 (Continued).

Parameter	Overall Sample (n=114)	
	Frequency (n)	Percentage (%)
AF Type		
Unknown	94	82.5
Paroxysmal AF	17	14.9
Permanent AF	1	0.9
Persistent AF	2	1.8
Category of drug used		
No antithrombotic therapy	4	3.5
OAC therapy	93	81.6
Antiplatelet therapy	17	14.9
Comorbidities		
Rheumatic heart Disease	26	22.8
COPD	29	25.4
Acute Kidney Injury	8	7.0
Other comorbidities	63	55.3
Congestive heart failure(Signs/symptoms of heart failure or objective evidence of reduced left ventricular ejection fraction)	35	30.7
Dyslipidemia	4	3.5
Carotid Artery Disease	6	5.3
Hypothyroidism	14	12.3
Hypertension(Resting BP >140/90 mmHg on at least two occasions or current antihypertensive treatment) fraction	45	39.5
Age (65–74 yrs)	38	33.3
Age ≥ 75yrs	33	28.9
Diabetes mellitus(Fasting glucose >125 mg/dL (7 mmol/L) or treatment with oral hypoglycemic agent and/or insulin)	16	14.0
Prior stroke, transient ischemic attack, or Thromboembolism	14	12.3
Stroke	12	10.5
Bleeding history/Predisposition(anemia)	9	7.9
Elderly (>65yr)	71	62.3
Antiplatelet therapy	15	13.2

Abbreviations: AF, atrial fibrillation; CHA2DS2VASC, congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, prior stroke or transient ischemic attack or thromboembolism (doubled), vascular disease, age 65 to 74 years, sex category; HASBLED, Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly (.65 years), Drugs/alcohol concomitantly; BMI, Body Mass Index; OAC, Oral Anticoagulants; COPD, Chronic Obstructive Pulmonary Disease.

HASBLED value without the labile INR was 0.96 (±0.775). Most patients had high CHA_2DS_2VASc scores, indicating a high risk of stroke, and moderate HASBLED scores, indicating a moderate risk of bleeding. The antithrombotic treatment distribution of 114 patients revealed that the majority of patients received anticoagulants (n=93, 81.6%), followed by antiplatelet therapy (n=17, 14.9%) and no therapy (n=4, 3.5%). A total of 74.6% had no formal education, 17.5% had education below SLC, and 7.9% had done SLC or above.

Most of the participants used rivaroxaban (n=51, 54.8%), more than one quarter used warfarin (n=34, 36.6%), and few used apixaban (n=8, 8.6%) (Table 2).

From Table 3, there was significant difference in age distribution between VKA and NOAC users (p=0.001). There was a significant difference in the proportion of patients aged >75 years (p=0.003), with more in the NOAC group (32.2%) than the VKA group (5.9%). Education levels differed significantly between groups (p=0.008). The NOAC group had a higher proportion of patients with no formal education (86.4%) compared to the VKA group (58.8%).

Table 2 Types of OACs Used (N=93)

OACs Type	n	%
Warfarin	34	36.6
Rivaroxaban	51	54.81
Apixaban	8	8.6

Abbreviation: OACs, Oral Anticoagulants.

Table 3 Characteristics of AF Patients Taking VKA and NOACs (N=93)

S.N.	Variables	Total OAC Sample (n=93)	VKA (n=34)	NOAC (n=59)	P value
1	Age, n (%)				
	18 to 64 years	37(39.8)	18(52.9)	19(32.2)	
	65 to 74 years	34(36.6)	14(41.2)	20(33.9)	0.007*
	≥ 75 years	22(23.7)	2(5.9)	20(33.9)	
2	Gender, n (%)				
	Female	58(62.4)	24(70.6)	34(57.6)	0.214
	Male	35(37.6)	10(29.4)	25(42.4)	
3	Education level, n (%)				
	No formal education	71(76.3)	20(58.8)	51(86.4)	
	Below SLC	15(16.1)	9(26.5)	6(10.2)	0.008*
	SLC and above	7(7.5)	5(14.7)	2(3.4)	
4	Employment, n (%)				
	Employed	5(5.4)	3(8.8)	2(3.4)	0.263
	Unemployed	88(94.6)	31(91.2)	57(96.6)	
5	Duration since diagnosis of AF, n (%)				
	Less than 2 years	32(34.4)	2(5.9)	30(50.8)	<0.001*
	More than 2 years	61(65.6)	32(94.1)	29(49.2)	
6	Smoking, n (%)				
	Yes	3(3.2)	1(2.9)	2(3.4)	0.666
	No	31(33.3)	9(26.5)	22(37.3)	
	Ex-smoker	59(63.4)	24(70.6)	35(59.3)	

(*Continued*)

Table 3 (Continued).

S.N.	Variables	Total OAC Sample (n=93)	VKA (n=34)	NOAC (n=59)	P value
7	Alcohol, n (%)				
	Yes	2(2.2)	1(2.9)	1(1.7)	1.000
	No	39(41.9)	14(41.2)	25(42.4)	
	Ex-drinker	52(55.9)	19(55.9)	33(55.9)	
8	CHA_2DS_2VASC risk, n (%)				
	Low risk (0)	5(5.4)	4(11.8)	1(1.7)	
	Intermediate risk (1)	19(20.4)	12(35.3)	7(11.9)	<0.001*
	High risk (≥2)	69(74.2)	18(52.9)	51(86.4)	
9	HASBLED risk, n (%)				
	Low risk (0)	32(34.4)	15(44.1)	17(28.8)	0.245
	Moderate risk (1–2)	60(64.5)	19(55.9)	41(69.5)	
	High risk (≥3)	1(1.1)	-	1(1.7)	
10	Duration since OAC use, n (%)				
	Less than 2 years	36(38.7)	2(5.9)	34(57.6)	
	More than 2 years	57(61.3)	32(94.1)	25(42.4)	<0.001*
11	RHD				
	No	69(74.2)	12(35.3)	57(96.6)	<0.001*
	Yes	24(25.8)	22(64.7)	2(3.4)	
12	COPD				
	No	74(79.6)	29(85.3)	45(76.3)	0.299
	Yes	19(20.4)	5(14.7)	14(23.7)	
13	Hypertension				
	No	58(62.4)	31(91.2)	27(45.8)	<0.001*
	Yes	35(37.6)	3(8.8)	32(54.2)	
14	Age (65–74 years)				
	No	41(44.1)	19(55.9)	22(37.3)	0.082
	Yes	52(55.9)	15(44.1)	37(62.7)	
15	Age (>75 years)				
	No	72(77.4)	32(94.1)	40(67.8)	0.003*
	Yes	21(22.6)	2(5.9)	19(32.2)	
16	Diabetes Mellitus				
	No	81(87.1)	32(94.1)	49(83.1)	0.125
	Yes	12(12.9)	2(5.9)	10(16.1)	
17	Prior Stroke				
	No	82(88.2)	29(85.3)	53(89.8)	0.514
	Yes	11(11.8)	5(14.7)	6(10.2)	
18	Bleeding History				
	No	86(92.5)	30(88.2)	56(94.9)	0.240
	Yes	7(7.5)	4(11.8)	3(5.1)	

Note: (*) indicates statistical significance with p<0.05 (95% confidence interval).

Abbreviations: AF, atrial fibrillation; VKA, Vitamin K antagonists; NOACs, Non Vitamin K Antagonist Oral Anticoagulants; CHA2DS2VASC, congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, prior stroke or transient ischemic attack or thromboembolism (doubled), vascular disease, age 65 to 74 years, sex category; HASBLED, Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly (. 65 years), Drugs/alcohol concomitantly; BMI, Body Mass Index; OAC, Oral Anticoagulants; RHD, Rheumatic Heart Disease; COPD, Chronic Obstructive Pulmonary Disease.

Table 4 Median(IQR) Knowledge Scores in Patients (N=93)

	Interquartile Range of Knowledge Scores		p value[a]
	VKA (n=34)	NOAC (n=59)	
General Knowledge (Section A)	14(11–17)	12(9–14)	0.001
Warfarin related knowledge (Section B)[b]	3.50(2.75–5)	–	–
General plus specific knowledge (Section A + B)	18(13–30)	12(9–14)	<0.001

Notes: [a]mann–Whitney *U*-test between VKA and NOAC groups. [b]Section B is only applicable to VKA group.
Abbreviations: IQR, Inter-quartile range; NOAC, Non-Vitamin-K-Antagonist Oral Anticoagulants; VKA, Vitamin K antagonist.

Likewise, there was a significant difference in the length of time since AF diagnosis (p<0.001). The majority of VKA users (94.1%) had been diagnosed for more than 2 years, compared to only 49.2% of NOAC users. CHA2DS2VASC scores differed significantly between groups (p<0.001). A higher proportion of NOAC users (86.4%) were categorized as high risk compared to VKA users (52.9%). The duration of OAC use differed significantly among the groups (p<0.001). While on comorbidities, RHD (p<0.001) and Hypertension (p=0.001) were found to have significant difference between the groups.

In the knowledge assessment, patients on VKA had higher median knowledge scores than those on NOACs did (p=0.001) (Table 4). Overall anticoagulation knowledge was inadequate (51.6%) (Figure 2).

When medication adherence was assessed, there were no significant differences in the median scores (p=0.193) between the two groups (Table 5). Medium (83.90%) and high adherence (16.10%) rates were reported by most patients (Figure 3).

From Table 6, while assessing for association between level of knowledge and adherence, there was no significant association seen between them.

According to the binary logistic regression, the duration since the use of oral anticoagulants was found to be associated with adequate knowledge (OR, 1.018; 95% CI, 1.002–1.033; p=0.026) (Table 7).

Similarly, for predictors of NOAC use, duration since the use of oral anticoagulants (OR, 1.01; 95% CI, 0.99–1.03; p=0.007) and hypertension (OR, 0.00; 95% CI, 0.00–0.44; p=0.023) were found to be associated with NOAC use (Table 8).

Discussion

This study aimed to assess oral anticoagulation knowledge and medication adherence among AF patients prescribed OACs in a cardiac outpatient department of a tertiary care hospital in Nepal. The demographic analysis revealed that the patient population had a high burden of comorbidities, with significant rates of hypertension (39.5%), COPD (25.4%), and congestive heart failure (30.7%). This demographic profile aligns with the literature, which indicates that AF predominantly affects older adults with multiple comorbidities.[25–27]

Figure 2 Distribution of Anticoagulation Knowledge.

Table 5 Medication Adherence Median(IQR) Scores in Patients (N=93)

	Interquartile Range of Adherence Scores		P value
	VKA (n=34)	NOAC (n=59)	
Adherence score	29(26.75–30)	29(28–30)	0.193

Abbreviations: IQR, Inter-quartile range; NOAC, Non-Vitamin-K-Antagonist Oral Anticoagulants; VKA, Vitamin K antagonist.

A total of 45 (48.4%) patients had overall adequate knowledge of anticoagulation, accounting for more than half of the patients who had inadequate knowledge. Considering the demographics, since most of the patients had no formal education, this might have resulted in gaps in their knowledge. The median anticoagulation knowledge score of those on warfarin was significantly higher than that of those on NOACs. Several possible explanations could account for this finding. Since warfarin requires frequent monitoring after the International Normalized Ratio (INR) test and dose adjustments if needed, these patients might have received comprehensive counseling. In contrast, those taking NOACs required less monitoring and had fewer visits to the hospital. These findings align with those of other studies conducted in various populations, where suboptimal knowledge of oral anticoagulants has been consistently reported. Our findings align with global studies highlighting gaps in anticoagulation knowledge among AF patients, yet reveal unique aspects specific to the Nepalese context. Similar to studies conducted in Australia and the United States, our research found suboptimal knowledge levels among AF patients, particularly those on NOACs, suggesting a need for consistent patient education across all anticoagulant types.[17,28–30]

A significant finding of this study was the association between the duration of OAC use and the level of anticoagulation knowledge. Patients with a longer duration of OAC use were more likely to have adequate knowledge about their oral anticoagulant therapy.[31] These findings suggest that ongoing education and reinforcement over time may improve patients' understanding of their treatment regimens.

Participants who are well versed in anticoagulation can make more informed decisions and manage their illness on their own. This study revealed that patients receiving NOACs have limited awareness of oral anticoagulants. It is critical to incorporate knowledge assessment into counseling programs and deliver it to patients with atrial fibrillation at the start of their oral anticoagulant therapy and on an ongoing basis to investigate and address an awareness gap. In the absence of

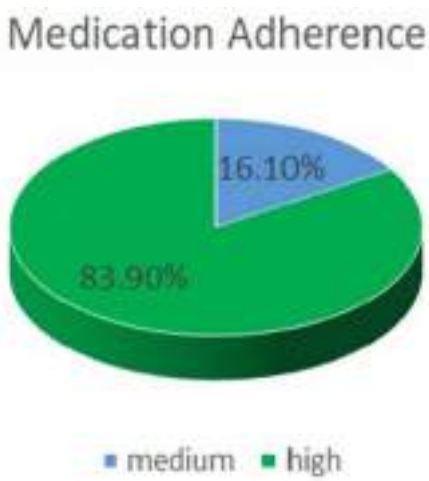

Figure 3 Distribution of Medication Adherence.
Abbreviations: AF, Atrial fibrillation; OACs, Oral Anticoagulants; LMICs, Low or middle income country; AKT, Oral Anticoagulation Knowledge Tool; ACDS, Chronic Disease Adherence Scale; NOACs, Non-Vitamin-K-Antagonist Oral Anticoagulants; VKA, Vitamin K Antagonists.

Table 6 Association Between Knowledge and Adherence

Variables		Level of Knowledge		Chi-square	p value
		Adequate Knowledge	Inadequate Knowledge		
Level of Adherence	Medium adherence	6	9	0.504	0.478
	High adherence	39	39		

Table 7 Factors Associated with Adequate Knowledge

S.N.	Odds of Adequate Anticoagulation Knowledge	Univariate Odds Ratio (95% CI)	p value	Multivariate Odds Ratio (95% CI)	p value
1	Age	0.95(0.91–0.99)	0.013*	0.98(0.92–1.03)	0.389
2	Gender (Female*/Male)	1.22(0.53–2.82)	0.649	–	–
3	Total Adherence	1.11(0.93–1.31)	0.253	–	–
4	Duration since use of Oral anticoagulants	1.02(1.01–1.03)	0.001*	1.018(1.002–1.033)	0.026*
5	Level of Education No formal education Below SLC SLC and Above*	 0.29(0.05–1.61) 0.80(0.11–5.68)	 0.158 0.823	 0.47(0.06–3.78) 0.99(0.10–9.56)	 0.478 0.991
6	Employment status (Employed*/Unemployed)	 4.59(0.49–42.69)	 0.181	–	–
7	Current Oral Anticoagulant taken by patient Warfarin* Rivaroxaban Apixaban	 0.31(0.12–0.77) 0.16(0.03–0.92)	 0.012* 0.040*	 1.48(0.24–9.06) 1.22(0.09–15.48)	 0.670 0.877
8	Rheumatic Heart Disease Yes*/No	 0.36(0.14–0.96)	 0.041*	 0.87(0.17–4.42)	 0.867
9	Chronic Obstructive Pulmonary Disease Yes*/No	 2.41(0.83–7.04)	 0.106	 1.86(0.48–7.29)	 0.373
10	Age>75 years Yes*/No	 5.62(1.72–18.38)	 0.004*	–	–
11	Duration since AF diagnosis	1.01(1.00–1.02)	0.002*	–	–
12	Diabetes mellitus Yes*/No	 2.38(0.66–8.53)	 0.184	 0.26(0.06–1.19)	 0.083
13	Bleeding History Yes*/No	 0.35(0.06–1.89)	 0.222	 0.30(0.04–2.32)	 0.250
14	Body Mass Index Yes*/No	 1.11(1.00–1.23)	 0.043*	–	–
15	CHA_2DS_2VASC risk Low risk Intermediate risk High risk*	 1.95(0.31–12.42) 2.23(0.78–6.35)	 0.480 0.133	–	–

Notes: (*) indicates odds ratio and adjusted odds ratio with 95%confidence interval is significant at p<0.05.
Abbreviations: AF, atrial fibrillation; CHA2DS2VASC, congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, prior stroke or transient ischemic attack or thromboembolism (doubled), vascular disease, age 65 to 74 years, sex category.

Table 8 Predictors of NOAC Use Over Warfarin

S.N.	Odds of NOACs Use	Univariate Odds Ratio (95% CI)	p value	Multivariate Odds Ratio (95% CI)	p value
1	Age of the patient	1.09(1.04–1.15)	0.001*	1.14(0.96–1.35)	0.146
2	Gender Female*/Male	1.76(0.72–4.34)	0.216	–	–
3	Duration since AF diagnosis	0.99(0.98–0.99)	0.001*	1.01(0.99–1.03)	0.487
4	Duration since use of Oral anticoagulants	0.94(0.91–0.99)	0.000*	0.86(0.77–0.96)	0.007*
5	Level of Education No formal education* Below SLC SLC and Above	 – 0.19(0.06–0.66) 0.139(0.025–0.789)	 – 0.009* 0.026*	 – 0.05(0.00–3.02) 0.02(0.00–2.68)	 – 0.150 0.118
6	Hypertension Yes*/No	 0.05(0.01–0.25)	 0.000*	 0.00(0.00–0.44)	 0.023*
7	Diabetes mellitus Yes/No*	 3.26(0.67–15.89)	 0.143	 –	 –
8	Rheumatic Heart Disease Yes/No*	 0.02(0.00–0.09)	 0.000*	 –	 –
9	CHA_2DS_2VASc risk Low risk Intermediate risk High risk*	 0.08(0.01–0.75) 0.18(0.060–0.55)	 0.028* 0.003*	 13.56(0.19–986.83) 1.43(0.04–45.13)	 0.233 0.838
10	HASBLED risk Low risk Intermediate risk High risk*	 0 0	 1.000 1.000	 –	 –
11	Body Mass Index	1.11(0.99–1.24)	0.055	–	–
12	Bleeding History Yes/No*	0.40(0.08–1.91)	0.252	–	–

Note: (*) indicates odds ratio and adjusted odds ratio with 95% confidence interval is significant at p<0.05.

Abbreviations: AF, atrial fibrillation; NOACs, Non Vitamin K Antagonist Oral Anticoagulants; CHA2DS2VASC, congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, prior stroke or transient ischemic attack or thromboembolism (doubled), vascular disease, age 65 to 74 years, sex category; HASBLED, Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly (. 65 years), Drugs/alcohol concomitantly.

frequent coagulation monitoring for NOACs, a comparable follow-up session could be developed for NOAC users to assess their understanding of oral anticoagulants and other patient-related outcomes.

During the assessment of medication adherence, most patients had high adherence scores. These findings are similar to studies performed across the world and in Nepal.[20,32] In contrast to studies where AF patients seem to be nonadherent to their medication, the context here is different.[33] The reason behind this might be the regular follow-up of the patients due to the health insurance policy of the hospital. The patients were provided medication for one month, and they had to revisit the doctor to prescribe their medication. Though the patients showed good adherence, since their knowledge seemed to be inadequate, they might lack essential information. This comparison shows that adopting international practices, such as providing consistent education for all patients, could improve safe and effective anticoagulation in Nepal. Tailored education programs could help close knowledge gaps and better support AF patients in managing their treatment.

To the best of our knowledge, this study is the first to compare anticoagulation knowledge between patients taking warfarin and those taking NOACs among AF patients in Nepal. This offers insights that could guide local clinical practice and inform future guidelines. The findings of this study contribute to the existing limited research on anticoagulation knowledge among AF patients prescribed OACs and further focus on interventions to improve this knowledge. Our study suggests that NOAC users often receive less education about their medication compared to warfarin users, possibly due to NOACs' simpler dosing and monitoring. This knowledge gap indicates that clinical practice could be improved by providing consistent education to all AF patients, regardless of the anticoagulant they use. Standardized educational protocols for all AF patients on medication risks and adherence, including routine sessions for NOAC users, could improve understanding and adherence, ultimately lowering risks of stroke and bleeding complications. Similarly, the findings of NOAC predictors of use among OAC patients enabled us to identify subjects who are more likely to be prescribed a NOAC, allowing us to focus interventions on promoting proper NOAC use.

This study underscores the importance of accessible and consistent patient education on anticoagulant therapy, regardless of medication type. Policymakers could use these findings to support initiatives that prioritize standardized patient education as a routine part of care for AF patients. Integrating such education into public health guidelines could improve patient outcomes, reduce preventable complications, and potentially lower healthcare costs associated with inadequate anticoagulation management. Our study highlights a knowledge gap between warfarin and NOAC users, adding evidence from Nepal to support the need for consistent anticoagulant education across settings.

Limitations

Our study has several limitations. First, the number of patients was small compared with that in other studies, probably due to the lower prevalence of AF. Second, as this was a single-center study, the findings could be generalizable. Third, among the patients who were found in adequate numbers, those who were not receiving OAC therapy, for whom questionnaire administration could not be performed, had to be excluded.

Future Directions

Future research should focus on testing different educational methods to find the most effective approaches. This could lead to guidelines that improve patient knowledge, support self-management, and reduce health risks across anticoagulant therapies. Future research should continue to explore the factors influencing anticoagulant use and the effectiveness of various educational strategies in improving patient knowledge and adherence.

Conclusion

Our findings reveal that AF patients on warfarin demonstrate greater knowledge of anticoagulation therapy compared to those on NOACs, likely due to the increased monitoring and counseling typically provided with warfarin. This knowledge gap highlights the need for consistent education across all anticoagulant types to improve patient understanding for better outcome. Enhanced education, especially for NOAC users, may support safer and more effective anticoagulation therapy. These conclusions are directly supported by the data, which showed significant differences in knowledge levels based on anticoagulant type.

Our study suggests that all AF patients, including those on NOACs, would benefit from standardized education on their medication. Clinicians could improve patient outcomes by ensuring consistent guidance on the risks and adherence needs for both NOAC and warfarin users.

In conclusion, addressing knowledge gaps and enhancing adherence through tailored educational interventions such as one-on-one counselling sessions, visual aids and pamphlets, mobile apps and reminders, follow-up calls, group education sessions are critical in optimizing anticoagulation therapy for atrial fibrillation patients.

Data Sharing Statement

The data supporting the findings of this study are available upon request from the corresponding author. However, the data are not publicly accessible, as they contain information that could compromise the privacy of the research participants.

Ethical Approval and Informed Consent

Ethical approval was obtained from the Institutional Review Committee of Kathmandu University School of Medical Sciences (IRC-KUSMS Approval Number: 73/24) which was in accordance with the Declaration of Helsinki. The participants in the study were informed about the purpose of the research and the significance of their involvement. Written informed consent was obtained from every participant before the survey was conducted.

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Author Contributions

All authors had significant contribution in the work from conception, study design, execution, and acquisition of data, data analysis, interpretation and manuscript writing. They all took part in revising and critically reviewing the article and approved the final version of the paper before submission to journal and agree to be accountable for all aspects of the work.

Disclosure

The author(s) report no conflicts of interest in this work. The author(s) have no relevant financial or nonfinancial interests to disclose. This study was conducted without any financial support or sponsorship from any organization or entity that could influence the results or interpretation of the findings.

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ORIGINAL RESEARCH

Elevated Vitamin B12 Levels in Myeloproliferative Neoplasm (MPN) Patients: A Potential Diagnostic and Prognostic Marker

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Background: Elevated vitamin B12 (B12) levels are linked to an increased risk of cancers, including hematological malignancies. This study focuses on the relationship between elevated B12 and myeloproliferative neoplasms (MPNs): Polycythemia Vera (PV), Primary Myelofibrosis (MF), Essential Thrombocytosis (ET), and Chronic Myeloid Leukemia (CML). Elevated B12 in MPNs is believed to arise from increased transcobalamin I (TCI) secretion by proliferating leukocytes, leading to higher serum levels. B12 may serve as a diagnostic and prognostic biomarker for these conditions. However, its sensitivity, specificity, and cutoff levels are unclear.

Aim: To assess the prevalence of high B12 levels in MPN patients, determine the median levels, identify a diagnostic cutoff, and evaluate the sensitivity and specificity of B12 as a marker.

Methods: Data were retrieved from the National Center for Cancer Care and Research in Doha, Qatar, for MPN patients from January 2016 to December 2022.

Results: A total of 467 patients were included: 232 with CML, 98 with PV, 88 with ET, and 50 with MF. The majority were male (66%) and of Asian origin (56%), with a median age of 48.7 years. CBC results showed median hemoglobin of 9.2 g/dL, WBC count of 73 x 10^3/uL, and platelet count of 531 x 10^3/uL. Elevated B12 levels were found in 95 patients (20%): 71% CML, 14% PV, 10% MF, and 5% ET. Extreme elevations were seen in 59 patients. The mean B12 level decreased from 747.3 ± 686.5 pg/mL before treatment to 397.9 ± 343.7 pg/mL after one year (p=0.01). Median levels were 458 pg/mL (718) before treatment and 301 pg/mL (229) after. In the extreme high B12 group, the mean was 1722 pg/mL before and 677 pg/mL after treatment.

Conclusion: Elevated B12 levels are associated with disease activity in CML. However, their role as a reliable marker for disease monitoring remains uncertain, and further studies are needed to confirm their utility for CML progression.

Keywords: Vitamin B12, myeloproliferative neoplasms, MPNs, chronic myeloid leukemia, CML, polycythemia vera, PV, essential thrombocythemia, ET, myelofibrosis, MF

Introduction

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of clonal hematopoietic disorders characterized by the abnormal proliferation of myeloid lineage cells, leading to a spectrum of clinical presentations, complications, and an increased risk of transformation to acute leukemia.[1] These disorders include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). Each MPN subtype is associated with specific genetic mutations—such as the *BCR-ABL* fusion gene in CML, or *JAK2, CALR*, and *MPL* mutations in BCR-ABL-negative MPNs.[2] These mutations, along with their distinct pathophysiological mechanisms, contribute to the diagnostic and therapeutic challenges that clinicians face in managing these diseases.[2]

In recent years, there has been growing interest in identifying reliable biomarkers that can enhance the accuracy of MPN diagnosis, improve risk stratification, and aid in monitoring disease progression. Traditionally, the diagnosis of

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MPNs has relied on a combination of clinical criteria, bone marrow histology, and genetic testing.[3] However, these methods may be insufficient to capture the full spectrum of disease activity, especially in early or atypical presentations. Peripheral blood biomarkers, including inflammatory markers, cytokines, and metabolic parameters, have been investigated as potential adjuncts to existing diagnostic tools.[4]

One such biomarker that has recently gained attention in hematologic malignancies is Vitamin B12. This essential water-soluble vitamin plays a key role in erythropoiesis and DNA synthesis. Elevated Vitamin B12 levels have been observed in several hematological disorders, including MPNs, where dysregulated metabolism of the vitamin may reflect underlying disease pathophysiology.[5] In MPNs, increased levels of Vitamin B12 are thought to result from the enhanced release of transcobalamin I (TCI) from proliferating white blood cells, which leads to higher serum levels of the vitamin.[6] Previous studies have suggested that elevated Vitamin B12 may correlate with disease activity, and may also serve as a marker of disease burden or progression in certain MPN subtypes, particularly PV and MF.[7]

Despite these findings, there is still limited data on the prevalence and clinical implications of elevated Vitamin B12 levels in MPNs, especially in different subtypes. Furthermore, there is a need to explore whether Vitamin B12 could serve as a reliable diagnostic and prognostic biomarker for these disorders.

The aim of this study was to investigate the prevalence and clinical significance of elevated Vitamin B12 levels in patients with MPNs at a tertiary cancer center in Doha, Qatar. Specifically, we sought to characterize the distribution of Vitamin B12 levels across different MPN subtypes, establish median values, and evaluate the potential of Vitamin B12 as a biomarker for MPN diagnosis and prognosis.

Methods

This retrospective cohort study was conducted using data from the National Center for Cancer Care and Research (NCCCR) in Doha, Qatar. All patients with a confirmed diagnosis of myeloproliferative neoplasms (MPNs) between January 2016 and December 2022 were eligible for inclusion. MPN subtypes considered in the study included chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). B12 levels were measured, with normal laboratory values defined as 156–596 pg/mL. Levels greater than 1000 pg/mL were designated as very high or extreme, levels exceeding 569 pg/mL as high, and levels below 145 pg/mL as low.

Study Population

Patients included in the study were diagnosed with MPNs according to the 2016 revised World Health Organization (WHO) diagnostic criteria, which include clinical features, hematological findings, bone marrow morphology, and the presence of specific genetic mutations (*BCR-ABL, JAK2, CALR, MPL*).[1] Patients with incomplete records or who lacked Vitamin B12 measurements were excluded from the study. No specific interventions were applied, and the study focused on observational data collected during routine clinical care.

Data Collection

Clinical data, including demographic information, laboratory results, bone marrow biopsy findings, genetic mutation status, and treatment history, were retrieved from the NCCCR's electronic medical record system. The database captures all relevant clinical, pathological, and laboratory data for patients managed at the center, and data were anonymized prior to analysis. For this study, Vitamin B12 levels and other clinical parameters were collected from the time of diagnosis (baseline values) and during follow-up visits if available.

Laboratory Assessments

Vitamin B12 levels were measured using chemiluminescent microparticle immunoassay (CMIA) at the NCCCR's laboratory. The lab's reference range for normal Vitamin B12 levels was 156–596 pmol/L. Vitamin B12 levels greater than 1000 pmol/L were defined as "very high" or "extreme" based on previous studies indicating that these elevated levels may have clinical significance in malignancies. Vitamin B12 levels were typically measured at the time of MPN diagnosis or during routine follow-up evaluations, including periods of active treatment. For patients with multiple Vitamin B12 measurements, the highest recorded value was used for analysis.

Sample Size Considerations

No formal sample size calculation was conducted prior to the study, as the analysis was retrospective, and all available patients diagnosed with MPNs between 2016 and 2022 were included. A total of [476] patients were identified from the database. The study aimed to include a large enough cohort to ensure meaningful statistical analysis, given the exploratory nature of the research.

Statistical Analysis

Statistical analyses were conducted using [software, eg, SPSS, R]. Descriptive statistics were used to summarize the distribution of Vitamin B12 levels across the various MPN subtypes. Median values were calculated for each MPN subtype, and differences between groups were assessed using appropriate statistical tests, such as the Kruskal–Wallis test for non-parametric data.

Receiver Operating Characteristic (ROC) curves were constructed to evaluate the predictive performance of elevated Vitamin B12 as a diagnostic and prognostic biomarker. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using various cut-off levels for Vitamin B12. A p-value of less than 0.05 was considered statistically significant.

Results

Study Population

The study included a total of 467 patients diagnosed with various myeloproliferative neoplasms: Chronic Myeloid Leukemia (CML) (n=232), Polycythemia Vera (PV) (n=98), Essential Thrombocythemia (ET) (n=88), and Myelofibrosis (MF) (n=50). The median age of the patients was 48.7 years, with a range from 18 to 89 years. Of the total patient population, 311 (66%) were male (see Table 1).

Table 1 Patient Characteristics (N=467)

Characteristic N (%), median [IQR]	**Level**	**Value**
AGE, years		48.7±14.5
GENDER	Male	311 (66.6%)
	Female	156 (33.4%)
ETHNIC	Asian	262 (56.1%)
	African	87 (18.6%)
	White	11 (2.4%)
	Others	107 (22.9%)
Disease Group	CML	232 (49.7%)
	ET	88 (18.8%)
	MF	50 (10.7%)
	PV	97 (20.8%)
WBC on diagnosis, median (IQR)		15.2 (8.6, 86.4)
HB, median (IQR)		12.6 (10.5, 14.5)

(*Continued*)

Table 1 (Continued).

Characteristic N (%), median [IQR]	Level	Value
PLT, median (IQR)		448.0 (261.0, 732.0)
B12 at diagnosis, median (IQR)		458.0 (290.0, 1008.0)
B12 after treatment, median (IQR)		301.0 (219.0, 448.0)

Abbreviations: CML, Chronic Myeloid Leukemia; ET, Essential Thrombocythemia; MF, Myelofibrosis; PV, Polycythemia Vera; WBC, White Blood Cell; HB, Hemoglobin; PLT, Platelet; B12, Vitamin B12; IQR, Interquartile Range.

Laboratory Findings

The laboratory findings revealed the following median values for hemoglobin, white blood cell (WBC) counts, and platelet counts:

- **Hemoglobin**: 12.4 g/dL
- **WBC Count**: 73 x 10^3/uL
- **Platelet Count**: 531 x 10^3/uL

Vitamin B12 Levels

Out of the 467 patients, 95 (20%) exhibited elevated levels of vitamin B12, with the distribution across diagnoses as follows:

- **CML**: 67 patients (71%)
- **PV**: 13 patients (14%)
- **MF**: 10 patients (10%)
- **ET**: 5 patients (5%)

Among the 95 patients with elevated B12 levels, extreme elevations were observed in 59 patients. The median vitamin B12 level for these patients prior to treatment was [insert median level] (range, [insert range]), which significantly decreased to [insert median level] (range, [insert range]) one year after initiating therapy (P=0.001).

The elevation of WBC counts in MPN is a significant mechanism underlying increased vitamin B12 levels, particularly observed in Philadelphia chromosome-positive CML. Notable reduction in vitamin B12 concentrations following treatment may serve as a potential indicator for assessing disease progression and treatment response.

White Blood Cell Counts

Comparative analysis of WBC counts at diagnosis revealed significant differences among the diagnostic groups. The mean WBC at diagnosis was highest in CML patients at 145.4 (SD=121.1), compared to:

- **ET**: Mean=9.6 (SD=4.1)
- **MF**: Mean=11.7 (SD=9.7)
- **PV**: Mean=12.3 (SD=7.1)

This difference was statistically significant, with a p-value <0.001. The median WBC counts also indicated significant variability, with CML showing the highest median WBC of 112.1 (IQR=47.0, 221.5), followed by:

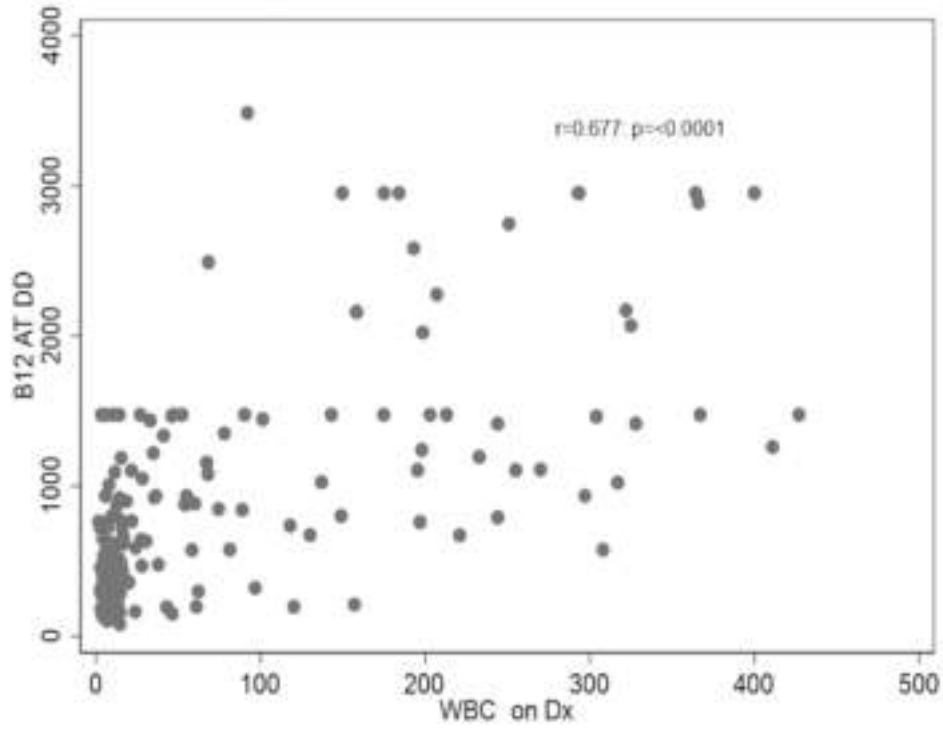

Figure 1 Correlation between vitamin B12 levels and white blood cell count.

- **PV**: Median=10.7 (IQR=7.5, 14.6)
- **MF**: Median=8.9 (IQR=6.4, 12.3)
- **ET**: Median=8.9 (IQR=7.2, 11.0)

Again, the p-value was <0.001, confirming statistical significance.

Correlation Between WBC Counts and Vitamin B12 Levels

Significant differences were also observed when comparing vitamin B12 levels at diagnosis across the groups. CML patients had the highest median vitamin B12 level of 1194.0 (IQR=760.0, 1476.0), followed by:

- **MF**: Median=392.0 (IQR=274.0, 616.0)
- **PV**: Median=373.0 (IQR=281.0, 527.0)
- **ET**: Median=290.0 (IQR=207.0, 424.0)

The p-value for these differences was <0.001. A correlation analysis between WBC count and vitamin B12 levels demonstrated a significant relationship, as illustrated in Figure 1.

Vitamin B12 Deficiency

Notably, only 9 patients (3.8%) were identified with vitamin B12 deficiency at the time of diagnosis.

Discussion

CML presents a distinct hematologic profile characterized by the presence of the Philadelphia chromosome and the fusion gene BCR:ABL1.[6] Although diagnosis traditionally hinges on the detection of these genetic abnormalities, emerging evidence suggests that peripheral blood biomarkers, such as vitamin B12 levels, could offer valuable insights into the pathophysiology and management of CML.[7] Our cohort analysis revealed a significant elevation in vitamin B12 levels among CML patients compared to other MPNs, exceeding 1000 pg/mL, indicating potential alterations in metabolic pathways and hematopoiesis.[8] Notably, post-treatment, there was a reduction in median vitamin B12 levels,

possibly correlating with disease burden and treatment response, emphasizing its dynamic nature in CML management.[9] While the precise mechanisms behind elevated vitamin B12 levels in CML remain elusive, hypotheses suggest disruptions in normal vitamin B12 homeostasis due to chronic myeloid proliferation and dysregulated hematopoiesis, or stimulation of cytokines and growth factors within the inflammatory microenvironment.[10,11]

CML patients often show significantly high cobalamin levels, sometimes even ten times above normal (Ermens et al, 2003). This increase is likely linked to the higher production of haptocorrin (HC) due to the greater number of leukocytes seen in CML. As HC is released from these cells, it picks up cobalamin from various tissues and the expanded granulocyte pool, leading to the elevated cobalamin levels observed in CML patients. Understanding why cobalamin levels rise in hematologic disorders sheds light on how these conditions work and could impact how we diagnose and treat them.[12]

Furthermore, recent studies have highlighted the importance of Vitamin B12 evaluation such as the assessment by Zeynelgil et al and the predictive value demonstrated by Ünlü et al, suggesting broader relevance across different hematological malignancies.[13,14] Elevated Vitamin B12 levels in CML not only aid in diagnosis but also extend to prognosis and monitoring, with potential implications for resource-limited settings where molecular testing is unavailable.[15,16]

In the context of solid malignancies, Vitamin B12 has garnered attention for its multifaceted roles in bodily functions. Research, such as the study by Urbanski et al (2020), has provided insights into the relationship between elevated Vitamin B12 levels and the risk of developing solid cancers.[17] This study's adjusted case-control analysis revealed intriguing findings regarding Vitamin B12 levels and the incidence of solid tumors, potentially offering valuable insights into its use as a biomarker for cancer risk and prognosis.[17] Moreover, evidence suggests that aberrant Vitamin B12 levels may serve as a prognostic indicator in solid tumors, with correlations to advanced disease stage and poorer clinical outcomes.[18] Supporting this notion, the study by Lacombe et al (2021) found a strong association between persistent elevation of plasma Vitamin B12 and the presence of solid cancer, emphasizing its potential utility as a diagnostic and prognostic marker in solid tumors.[19] Despite incomplete understanding of the precise mechanisms underlying Vitamin B12 dysregulation in solid malignancies, ongoing research endeavors aim to elucidate its role in tumorigenesis and explore its potential utility as a therapeutic target or prognostic marker.

Research increasingly suggests a link between low Vitamin B12 levels and cancer, with potential implications for cancer risk and prognosis. For example, a study by de Souza et al in 2020 found a significant connection between Vitamin B12 deficiency and various cancers, including gastrointestinal and hematological malignancies.[20] Low Vitamin B12 levels have been associated with changes in DNA synthesis, weakened DNA repair mechanisms, and compromised immune function, contributing to cancer development and progression.[20] Additionally, a prospective study by Miranti et al in 2017 highlighted the increased risk of gastric cancer associated with low Vitamin B12 levels,[21] underscoring the importance of maintaining optimal Vitamin B12 levels for cancer prevention. These findings emphasize the significance of maintaining adequate Vitamin B12 levels for reducing cancer risk and improving clinical outcomes. In our population, we have found very low incidence of vitamin B12 deficiency underscores the rarity of true deficiency in these patients.

Research has emphasized the importance of reflective molecular testing for MPNs in patients with elevated serum Vitamin B12 levels. This approach, elucidated in previous studies,[22] underscores the necessity of a comprehensive diagnostic strategy to understand the underlying causes of elevated Vitamin B12 levels, particularly concerning hematological malignancies. By integrating molecular testing alongside traditional diagnostic methods, clinicians can uncover the genetic landscape of MPNs, including mutations in genes like JAK2, CALR, and MPL, which provide crucial insights into disease development and risk assessment. Moreover, reflective molecular testing assists in identifying potential therapeutic targets and guides personalized treatment decisions, improving the precision and effectiveness of patient care strategies. This integrated approach highlights the evolving landscape in MPN diagnostics, emphasizing the synergy between molecular insights and clinical practice to optimize patient care and outcomes.[22] Therefore, it can be used along with molecular to establish diagnosis and differentiate primary from secondary polycythemia and so on.

Furthermore, studies such as those by Gilbert et al[23] and Cinemre et al[24] have contributed to our understanding of serum Vitamin B12 content and its implications in myeloproliferative diseases. These investigations underscore the value of Vitamin B12 assessment in differential diagnosis and as indicators of disease activity, providing clinicians with valuable tools for disease management and prognosis.

In summary our cohort highlighted the increase in WBC as one of the important mechanism behind elevated vitamin B12 in MPNs, and therefore it was more prevalent in Philadelphia positive CML. Significant change of vitamin B12 observed after treatment could possibly be used for follow up.

Conclusions

This study highlights the epidemiology and clinical characteristics of myeloproliferative disorders (MPNs) in a cohort of 467 patients, predominantly male and with significant representation of individuals of Asian origin. Key findings include elevated vitamin B12 levels, particularly in CML patients, which significantly decreased following treatment. The observed correlation between vitamin B12 levels and white blood cell count at diagnosis suggests a potential interplay between these factors. These insights may inform the use of vitamin B12 as a diagnostic and follow-up biomarker, guiding future research and therapeutic strategies to enhance patient care and outcomes.

Statement of Ethics

This research was approved by the Hamad Medical Corporation's Medical Research Center. The ethics committee waived the requirement for consent because our research did not include any names or any data regarding patient identity. The study complies with the Declaration of Helsinki.

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Disclosure

The authors have no conflicts of interest.

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