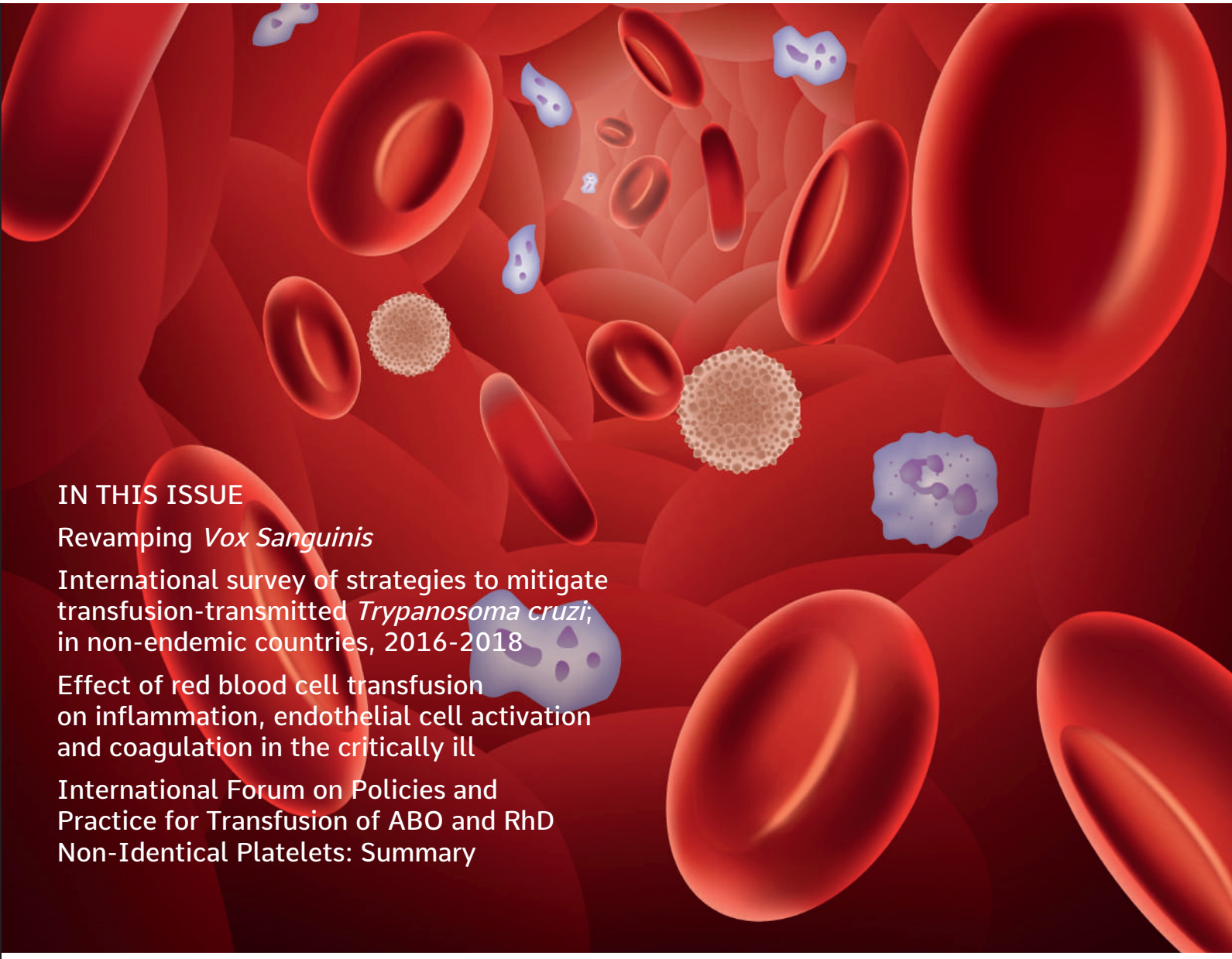
ISSN 0042-9007 (Print)
ISSN 1423-0410 (Online)

Volume 117 Number 1 JANUARY 2022

Vox Sanguinis

The International Journal of Transfusion Medicine



IN THIS ISSUE

ISBT International Society of Blood Transfusion

WILEY

Vox Sanguinis

International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

Founded 1956 by J. J. van Loghem, L. P. Holländer, J. Dausset, A. Hässig and J. Julliard (formerly Bulletin of the Central Laboratory of the Blood Transfusion Service of the Dutch Red Cross, founded 1951)

Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

Vox Sanguinis reports on all issues related to transfusion medicine, from donor vein to recipient vein, including cellular therapies. Comments, reviews, original articles, short reports and international fora are published, grouped into 10 main sections:

1. Blood Component Collection and Production: Blood collection methods and devices (including apheresis); blood component preparation; inventory management; collection and storage of tissues; quality management and good manufacturing practice; automation and information technology; plasma fractionation techniques and plasma derivatives;
2. Cellular Therapy: Cell-based therapies; CAR T-cell therapies; genetically modified cell therapies; cellular therapy (sources; products; processing and storage); stem cells; cell-based regenerative medicine; cellular immunotherapy; molecular therapy;
3. Donors and Donations: Donor recruitment and retention; donor selection; donor health
4. Haemovigliance: Adverse events in blood and blood component donors and transfusion recipients; corrective and preventive measures of complications; near-misses and errors in the transfusion chain; evaluation and outcomes of adverse events
5. Immunohaematology and Immunogenetics: autoimmunity in haematology; alloimmunity of blood; pre-transfusion testing; complement in immunohaematology; blood phenotyping and genotyping; genetic markers of blood cells and serum proteins: polymorphisms and function; parentage testing and forensic immunohaematology;
6. International Forum: Section in which topics related to any aspects of the transfusion chain (from technical to scientific, including socioeconomic and ethical facets) are discussed by invited participants and summarized by guest editors
7. Patient Blood Management: Bloodless surgery; preoperative anaemia; minimizing blood loss during surgery; alternatives to blood transfusion;
8. Review: Comprehensive reviews or short comments of any aspect related to transfusion medicine;
9. Transfusion Medicine: Transfusion indication, transfusion practice, thresholds and audits; transfusion efficacy assessment, clinical trials; therapeutic apheresis;
10. Transfusion-transmitted Disease and its Prevention: Identification and epidemiology of infectious pathogens transmissible by blood; donor testing for transfusion-transmissible infectious pathogens; bacterial contamination of blood components; pathogen inactivation;

This comprehensive coverage has made the journal essential reading for a wide range of specialists interested in the present state of transfusion research and practice.

Journal Customer Services

For ordering information, claims and any enquiry concerning your journal subscription please go to https://hub.wiley.com/community/ support/onlinelibrary or contact your nearest office. Americas: Email: cs-journals@wiley.com; Tel: +1 781 388 8598 or +1 800 835 6770 (toll free in the USA & Canada). Europe, Middle East and Africa: Email: cs-journals@wiley.com; Tel: +44 (0) 1865 778315. Asia Pacific: Email: cs-journals@wiley.com; Tel: +65 6511 8000. Japan: For Japanese-speaking support, Email: cs-japan@wiley.com. Visit our Online Customer Help at https://hub.wiley.com/community/support/ onlinelibrary.

Information for Subscribers: *Vox Sanguinis* is published in 12 issues per year. Institutional subscription prices for 2022 are: Print & Online: US$2196 (US), US$2560 (Rest of World), €1533 (Europe), £1189 (UK). Prices are exclusive of tax. Asia-Pacific GST, Canadian GST/HST and European VAT will be applied at the appropriate rates. For more information on current tax rates, please go to www. wileyonlinelibrary.com/ tax-vat. The price includes online access to the current and all online backfiles to 1 January 2018, where available. For other pricing options, including access information and terms and conditions, please visit www.wileyonlinelibrary.com/access.

Delivery Terms and Legal Title: Where the subscription price includes print issues and delivery is to the recipient's address, delivery terms are Delivered at Place (DAP); the recipient is responsible for paying any import duty or taxes. Title to all issues transfers FOB our shipping point, freight prepaid. We will endeavour to fulfil claims for missing or damaged copies within six months of publication, within our reasonable discretion and subject to availability.

Back issues: Single issues from current and recent volumes are available at the current single issue price from cs-journals@wiley.com. Earlier issues may be obtained from Periodicals Service Company, 351 Fairview Avenue – Ste 300, Hudson, NY 12534, USA. Tel: +1 518 822-9300, Fax: +1 518 822-9305, Email: psc@periodicals.com

Abstracting and Indexing Services: The Journal is indexed by Abstracts in Anthropology (Sage); Abstracts on Hygiene & Communicable Diseases (CABI); Academic Search (EBSCO Publishing); Academic Search Alumni Edition (EBSCO Publishing); Academic Search Premier (EBSCO Publishing); AGRICOLA Database (National Agricultural Library); BIOBASE: Current Awareness in Biological Sciences (Elsevier); Biological Abstracts (Clarivate Analytics); BIOSIS Previews (Clarivate Analytics); CAB Abstracts® (CABI); CABDirect (CABI); CAS: Chemical Abstracts Service (ACS); CSA Biological Sciences Database (ProQuest); CSA Environmental Sciences & Pollution Management Database (ProQuest); CSA Virology & AIDS Abstracts (ProQuest); Current Contents: Life Sciences (Clarivate Analytics); Embase (Elsevier); Global Health (CABI); HEED: Health Economic Evaluations Database (Wiley-Blackwell); Index Veterinarius (CABI); Journal Citation Reports/Science Edition (Clarivate Analytics); MEDLINE/PubMed (NLM); Nutrition Abstracts & Reviews Series A: Human & Experimental (CABI); Pig News & Information (CABI); ProQuest Central (ProQuest); ProQuest Health & Medical Complete (ProQuest); ProQuest Research Library (ProQuest); Protozoological Abstracts (CABI); PubMed Dietary Supplement Subset (NLM); Review of Medical & Veterinary Entomology (CABI); Review of Medical & Veterinary Mycology (CABI); Rural Development Abstracts (CABI); Science Citation Index (Clarivate Analytics); Science Citation Index Expanded (Clarivate Analytics); Tropical Diseases Bulletin (CABI); Veterinary Bulletin (CABI).

VOX SANGUINIS (Online ISSN: 1423-0410 Print ISSN: 0042-9007) is published monthly. US mailing agent: Mercury Media Processing, LLC, 1850 Elizabeth Avenue, Suite #C, Rahway, NJ 07065 USA. Periodical postage paid at Rahway, NJ. Postmaster: Send all address changes to VOX SANGUINIS, John Wiley & Sons Inc., C/O The Sheridan Press, PO Box 465, Hanover, PA 17331, USA. For submission instructions, subscription and all other information visit: www.wileyonlinelibrary.com/journal/vox. Printed in Singapore by C.O.S. Printers Pte Ltd.

Vox Sanguinis (2022) **117**, 1–152

117/1/2022

Contents

WILEY

This journal is available online at *Wiley Online Library*. Visit wileyonlinelibrary.com to search the articles and register for table of contents e-mail alerts.

Contents

Received: 10 December 2021 | Accepted: 10 December 2021

DOI: 10.1111/vox.13234

EDITORIAL

Vox Sanguinis ISBT International Society of Blood Transfusion

Revamping *Vox Sanguinis*

The role of *Vox Sanguinis* ('the voice of blood' in Latin) as the official journal of the International Society of Blood Transfusion (ISBT) began in January 1956, although, in fact, the journal had already existed under that name since 1953. During the 66 years of this role as 'Official Journal', with the publication of approximately 6000 papers, *Vox* has borne witness to the enormous changes that the field of haemotherapy has undergone: from the recruitment and management of donors, to the discovery of the infectious diseases that can be transmitted through transfusion; from the improvements in the preparation and storage of blood components to the routine use of platelet concentrates in thrombocytopaenic patients. A more poignant role that *Vox Sanguinis* has also performed over the course of these years has been to announce the deaths of colleagues and, at the same time, pay tribute to them for the contributions they have made to the advancement of our field. Obituaries for colleagues such as Alexander S. Wiener, Robert R. Race, Philip Levine, Tibor J. Greenwalt, Claës F. Hogman and, sadly, in this issue, Anneke Brand, have been published in *Vox*. Interestingly, of the 36 obituaries published, five were for women, but if we count only the last 4 years, they represent 40%.

Over the years, the cover and design of *Vox* have been modified on several occasions. One such modification coincided with the change of publisher from Karger AG to Blackwell (later Wiley-Blackwell) in January 2001, while another was to commemorate the 100th volume of *Vox* in January 2011. Now, 11 years on, we have decided that it is time to make changes again. This time, as readers of the paper-based issues can see, on the journal's front cover we have decided to include images, on a red background, of the three types of blood cell in circulation. As you read this, you will probably have already noticed another change we have implemented in the journal articles: a new layout. In this new layout, the red colour is present again, but more subtly, in the headings, in the shading of the abstract and in the tables. We felt that, since the journal is the 'voice of blood', the colour of blood ought to be present both on the cover and in the articles we publish. The perceptive reader will also have noted another subtle change; in contrast to the previous design, in the journal logo, next to the ISBT logo at the top of each page, now the word that is set in red is 'Sanguinis' and not 'Vox' because, we reasoned, this is exactly as it is in reality.

We are also happy to announce that other formal changes have been implemented in the journal. One is that, with the addition of a scientific/medical illustrator to the Editorial Team (Ms. Alison Schroeer, www.illustratingscience.com), we can now supplement those review articles that cover a suitable topic with colour artwork free of charge to the authors. Readers will also notice that there will be more colour illuminating the papers we publish because our publisher, Wiley, has ceased to apply colour charges for the figures and graphs that summarize the data presented in each paper.

There will be further changes regarding the journal's Editorial Team. Two new sections have been created, Haemovigilance and Patient Blood Management, which are two topics that are increasingly important in the field of haemotherapy. Two well recognized colleagues will head up these two new sections of Vox: Dr Claudia S. Cohn will be responsible for the new Haemovigilance section and Dr Nelson Tsuno for the Patient Blood Management section. In addition, a group of haemotherapy experts, covering all fields of transfusion medicine, from 24 different countries, have agreed to join our Section Editors to create a really comprehensive Editorial Board, thus, cementing the position of *Vox Sanguinis* as a truly international journal. We are extremely happy to highlight that the three most recent Editors-in-Chief for *Vox*, Prof. Dame Marcela Contreras, Prof. Wolfgang R. Mayr and Prof. Dana Devine, and the ex-Editor-in-Chief of the *ISBT Science Series* journal, Dr Pieter van der Meer, have kindly agreed to be part of this new expanded Board.

More changes will appear throughout the year. One is that there will be an increased number of issues. The Editorial Team is grateful to the ISBT Standing Committee on *Vox Sanguinis*, supported by the ISBT Board of Directors, for approving the proposal to increase the number of issues published each year to 12, so an issue of *Vox Sanguinis* will come off the press every month. Yet another change is already noticeable in this first issue of 2022, which is the increase in the number of pages published. The ISBT has approved the aim of reducing the time from acceptance of a paper to publication in an issue, to around 3 months. This is why, in order to decrease the current backlog, a thicker version of *Vox Sanguinis* will be delivered to your desktop in each of the first 3 months of 2022.

All these changes have only one objective and that is to maintain the position of *Vox Sanguinis* as one of the main reference journals in the transfusion medicine field and, if possible, improve the attractiveness of the journal to the international authors who look for the best platform on which to publish their work. The Editorial Team has worked hard to provide that platform. Now, our desire and hope are that the international blood transfusion community will take full advantage of it.

Miquel Lozano

Editor-in-Chief, Vox Sanguinis, Barcelona, Catalonia, Spain

Email: mlozano@clinic.cat

ORCID

Miquel Lozano https://orcid.org/0000-0003-2593-833X

Received: 26 January 2021 | Revised: 9 April 2021 | Accepted: 29 April 2021

DOI: 10.1111/vox.13175

REVIEW ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Use of plerixafor to mobilize haematopoietic progenitor cells in healthy donors

Iñigo Romon[1] | Carlos Castillo[2] | Joan Cid[2] | Miquel Lozano[2]

[1]Transfusion Service, Hematology and Hemotherapy Service, University Hospital Marqués de Valdecilla, IDIVAL, Santander, Spain

[2]Apheresis & Cellular Therapy Unit, Department of Hemotherapy and Hemostasis, ICMHO, Hospital Clínic, IDIBAPS, UB, Barcelona, Spain

Correspondence
Joan Cid, Unitat d'Afèresi i Teràpia Cel·lular, Servei d'Hemoteràpia i Hemostàsia, ICMHO, Hospital Clínic, C/. Villarroel, 170, 08036 Barcelona, Catalonia, Spain.
Email: jcid@clinic.cat

Abstract

Increased transplant activity calls for improved stem cell collection, especially when peripheral blood is the preferred source of haematopoietic progenitor cells (HPCs). Plerixafor is a bicyclam molecule that mobilizes CD34+ cells by reversibly disrupting CXCR4-CXCL12-supported HPC retention. Plerixafor is given with granulocyte colony-stimulating factor (G-CSF) to help harvest autologous CD34+ cells for transplantation when mobilization with G-CSF fails. Mobilization protocols with the same doses of plerixafor and G-CSF have been used off-label in healthy allogeneic donors, with equal success and scarce side effects, both in adult and paediatric patients. Plerixafor has also been used as a sole mobilization agent. Plerixafor alone or coupled with G-CSF might lead to harvesting distinct cellular populations conferring improved engraftment properties and increased survival. Those characteristics might make plerixafor an especially attractive mobilization agent, particularly for non-related donations. However, available data are limited, and long-term follow-up is needed to clarify the best scenario for using plerixafor with or without G-CSF in healthy donors. In this review, we will summarize the evidence supporting this practice, highlighting the practical aspects and providing clues for an expanded use of plerixafor.

KEYWORDS
apheresis–donation, cellular therapy, donor health, hematopoietic stem cell, peripheral blood stem cell, stem cell

INTRODUCTION AND OBJECTIVES

All modalities and clinical indications of haematopoietic cell transplantation (HCT) have grown during the last 50 years. In 2019, the European Society of Blood and Marrow Transplantation (EBMT) recorded over 47,000 HCTs. Allogeneic HCTs have expanded faster than autologous transplants, even more intensely for paediatric patients [1]. According to the World Marrow Donor Association (WMDA), non-related transplants have grown from 4,429 in 2000 to 23,181 in 2019 [2].

While bone marrow (BM)-derived donations confer a lower rate of chronic graft versus host disease (GVHD) after allogeneic HCTs, donation of haematopoietic progenitor cells (HPC) from peripheral blood (PB) results in a faster neutrophil and platelet engraftment and potentially shorter hospital stay and less transfusion support [3]. Therefore, PB is the preferred source of HPCs, accounting for 99% of autologous transplants and 76% of allogeneic donations (85% among unrelated donors) recorded by EBMT [1]. A similar historic shift among allogeneic donations was recorded by the Center for International Blood and Marrow Transplant Research [4]. Among non-related transplants, the evolution is also striking: according to WMDA, in 2000, BM comprised 3,514 donations (79%) for 4,429 transplants, while, in 2020, it amounted to only 3,914 (17%) donations for 23,181 transplants. Meanwhile, PB climbed from 24% donations in 2000 (1049 of 4429) to 68% in 2020 (16,406 of 23,181) [2].

BM harvest from healthy donors (HDs) has several disadvantages, being an invasive procedure subject to the dangers of general anaesthesia and the logistic demand of a surgical theatre, anesthesiology and haematological teams [5]. Moreover, as less BM collections take place, hospitals find it more difficult to maintain competent harvesting teams, making it harder to obtain adequate BM products. A recent study found that while BM volumes collected increased with time, cell concentrations decreased, putting both donors and patients at risk of adverse outcomes [6]. As higher nucleated cell doses are associated with less transplant-related mortality and improved survival after related and unrelated donor HCT, the difficulty in obtaining high-quality BM products could in turn skewer the balance even more towards PB harvests. Apart from the economical and logistical costs inherent to BM harvesting, HDs refer fatigue, weakness and other negative effects on quality of life several weeks after donation [7]. Besides, autologous whole blood donation is generally collected before BM harvesting.

On the contrary, collection of HPCs from PB is performed with an apheresis device using preferably peripheral veins that can be managed by nursing staff in an outpatient environment [8, 9]. This makes PB a more accessible and convenient source of HPCs for transplantation [5].

HPCs are recognized with the CD34 membrane marker. In physiological conditions, CD34+ cells are located in the BM, and a very small fraction (10–100 CD34+ cells/ml) is present in PB [10–12]. A mobilization regime is needed to shift HPCs from BM niche to PB. Autologous HPC donations rely on granulocyte colony-stimulating factor (G-CSF) as a mobilization agent, but up to 40% of autologous mobilization attempts fail to collect enough CD34+ cells for transplantation [13], and plerixafor (Mozobil™, Bridgewater, NJ) is used as an adjuvant [14, 15].

In the allogeneic setting, mobilization regimes of PB HPCs also rely on G-CSF. Successful mobilization rates among HDs vary greatly according to different authors. Beelen et al., in 2002, reported up to 25% failures to obtain ≥5 × 10^6 CD34+ cells/kg of recipient body weight in a single leukocytapheresis product after administering G-CSF at a dose of 16 μg/kg per day [16]. More recent works, some including children, reported that 2%–11% of G-CSF-based mobilization procedures fail to obtain the desired amount of CD34+ cells [17–19]. Use of plerixafor in this setting is considered off-label, as plerixafor is licensed by European Medicines Agency in Europe [20] and by the Food and Drug Administration (FDA) in the United States [21] only in combination with G-CSF for CD34+ cell mobilization failures for autologous HCT in lymphoma and myeloma patients. As a result, evidence on the use of plerixafor as a mobilization agent in HDs for allogeneic HCT is scarce [22–32].

The ideal mobilization regime should be fast, not needing several days of injections, preferably only one dose given the same day the apheresis is planned; efficient, obtaining a predictable and strong mobilization allowing collection of target CD34+ cell amount in a single apheresis session; with less adverse effects that actual regimes; yield a HPC product that results in a quicker haematopoietic engraftment, resulting in fewer infections, bleeding and potentially hospital stay and desirably with a lower rate of acute and chronic GVHD [33].

In this paper, we will review the evidence on the use of plerixafor as a mobilizing agent in HDs for allogeneic transplantation, with a special interest on practical clinical aspects.

WHAT IS PLERIXAFOR?

Plerixafor (formerly known as AMD3100) is a small bicyclam molecule that reversibly antagonizes the union between C-X-C motif chemokine receptor 4 (CXCR4, CD184), located on CD34+ cell membrane, and C-X-C motif chemokine 12 (CXCL12), located on the stromal cell membrane, via selective blockade of CXCR4 [34–36]. The disruption of CXCR4-CXCL12-supported HPC retention in the BM niche results in migration of HPCs to PB.

CXCR4, a member of 7-transmembrane G-protein-coupled receptors family, is expressed not only on HPCs but also on endothelial cells, microglia, lymphocytes, stromal fibroblasts and cancer cells [37]. Therefore, the CXCR4/CXCL12 axis regulates tumour angiogenesis and metastasis, mediates immune dysfunction and plays critical roles in many other biological processes [38].

CXCL12, formerly known as stromal cell–derived factor 1 (SDF-1), is a chemokine expressed in many normal and cancer cells. CXCL12 located on stromal cells binds to CXCR4 in CD34+ cells to activate downstream signalling complexes involving cellular processes including cell proliferation, migration and differentiation [38].

The use of plerixafor as a CD34+ cell mobilization agent was serendipitously discovered while researching for human immunodeficiency virus (HIV) medications, as plerixafor blocks HIV entry into T cells [34]. Initially considered an 'impurity' among the cyclam products under study, it showed a higher antiviral activity than other compounds. Plerixafor finally did not qualify as antiviral due to its adverse cardiac effects at antiviral doses, its lack of oral bioavailability and its inability to block macrophage-tropic HIV strains [39]. During the pharmacokinetic studies of plerixafor in HIV-1 phase-I clinical trials, a rapid increase in white blood cell (WBC) counts was observed in PB, even at a low dosage of the drug. The increase peaked about 6 h following the intravenous injection of plerixafor [34]. Liles et al. performed some of the earliest studies to verify that increased WBCs included a high number of CD34+ cells in healthy volunteers and that plerixafor acted synergistically with G-CSF in mobilizing CD34+ cells from BM into PB circulation, improving the mobilization and apheresis yield, which suggested that the new agent could be used in mobilization regimes for HCT purposes [40, 41]. Later, a pilot trial suggested that plerixafor could mobilize enough functional CD34+ cells from healthy related donors for the reconstitution of haematopoiesis in patients who received myeloablative allogeneic HCT [22]. More recently, some authors suggested that plerixafor could be the optimal mobilization agent for HDs [5].

HOW IS PLERIXAFOR USED? DOSING AND ADMINISTRATION

Plerixafor has been used with HDs as a mobilization salvage agent after mobilization failure or when failure is expected [42]. Like in autologous transplantation, three different strategies have been defined in the use of plerixafor: 'delayed re-mobilization' (when a previous mobilization cycle has failed), 'up front' (when a mobilization failure is expected) and 'pre-emptive use' [43].

Pre-emptive use, also known as 'rescue' or 'just in time use', refers to the introduction of plerixafor in the course of a mobilization episode in order to improve the collection of HPCs and is usually guided by the scarce number of CD34+ cells circulating in PB before starting leukocytapheresis (usually <10,000 cells/ml) [44, 45]. Up front and pre-emptive use rely on the prediction of a poor mobilization. Predictive factors for poor mobilization in HDs are still to be fully understood, usually rely on experience from autologous donors, which are usually inaccurate [11, 13]. Teipel et al., in a retrospective study of 7,216 HDs, found that female sex, older age, smoking, elevated lactate dehydrogenase, higher lymphocyte count and large unstained cell count were associated with poor CD34+ cell count after 4 days of mobilization with G-CSF [17]. Kimura et al. analysed a series of 109 allogeneic donors and 181 leucocytapheresis procedures, and they found that donors' cytomegalovirus (CMV) seronegative status and male gender yielded better results [46]. In the same study, the best yields happened in donors between 20 and 30 years old, with yields decreasing in donors older than 40 years. Kong et al. analysed 175 donors, found 39% failure rate, where older age (i.e., older than 55 years), a lower platelet count (i.e., < 210 $\times$ 10^9/L) and lower CD34 + cell in PB (i.e., <25,000/ml) were the main factors related with failed mobilization [47]. Sadly, no validated score exists to help predict mobilization failure.

In paediatric transplantation, an adverse donor/recipient weight ratio (<0.75) can appear in 33% donor/recipient pairs and is usually a source of special concern [29]. Other authors analysed 29 HDs weighing ≤20 kg, where a third of subjects had a donor/recipient weigh ratio ≤ 1. All donors achieved the desired CD34+ cell amount. However, only one-third of cases succeeded with one apheresis session, while 20% of them needed three apheresis procedures to achieve the desired amount of CD34+ cells [48]. Truong et al. described an 11% failure rate (4 out of 35 HDs), and only older age was found significant, although older age correlated with lower CD34 + cell count pre apheresis, being ≥20,000/ml the ideal value [19]. In a small series with a median age of 16 years, authors found that age was the only significant predictor of successful mobilization [49].

When administration of plerixafor to HDs is decided, several caveats should be considered. Dose, route of administration and timing from administration to leukocytapheresis start can be learned from previous published experiences (Table 1) as well as adverse events [22–25, 27, 28, 50–53].

First, the most commonly reported dose of plerixafor administered to HDs is 0.24 mg/kg, but some authors have tried lower or higher doses of plerixafor after G-CSF failure or using plerixafor alone [24, 25]. In an attempt to overcome mobilization failures, Pantin et al. conducted a trial in healthy volunteers comparing standard and double doses of plerixafor (0.24 vs. 0.48 mg/kg given subcutaneously). Double doses obtained a roughly 10% increase in peak CD34+ cell count and an increase in the area under the curve, with improved results in poor mobilizers. Another finding was that peak CD34+ cell count was delayed by several hours. However, the trial did not perform apheresis. Adverse effects, especially neurological and intestinal, increased slightly, but no subject suffered over grade-2 effects [54].

Second, subcutaneous injection is the most frequent way of administration, but one group designed a phase I/II study to evaluate the safety and efficacy of administering a single intravenous plerixafor administration. Authors administered plerixafor at a range dose of 0.08–0.48 mg/kg, concluding that the maximally effective dose was 0.32 mg/kg, and they did not observe dose-limiting toxicities with intravenous administration of plerixafor [25].

Finally, timing from plerixafor administration to start of leukocytapheresis ranged from 4 to 11 h (Table 1). When published, the median increase of CD34+ cells in PB before and after plerixafor administration ranged from 2 to 3, but there was a significant donor-to-donor variability (Table 1). Recently, Hölig et al. found that CD34+ yield increased up to five times, especially in donors with poor mobilization [30]. Apheresis is usually performed on day +5 of mobilization with G-CSF. However, one group took advantage of the fact that the increase in CD34+ cells is variable, finding that up to a third of allogeneic donors mobilized efficiently by day +4 of mobilization, sparing the donor side effects [55].

Success of a mobilization cycle also depends on apheresis collection efficiency [56]. High-volume apheresis protocols are commonly used for autologous HPC collection. However, in the allogeneic setting, apheresis procedures should be planned, customized and performed on the day of maximum CD34+ cell count in PB to maximize HPC yield. Facilities performing apheresis should know their collection efficiency and should use the best-suited apheresis protocols to tailor the process to the expected CD34+ cell yield, thus reducing the number of apheresis procedures needed and minimizing inconvenience and adverse effects to the donor [13, 44, 54]. Bartnik et al. in a series of 80 donors found that continuous mononuclear collection protocol using a Spectra Optia platform (TerumoBCT, Leuven, Belgium) impacted favourably in donors with negative collection predictors [57].

In paediatric patients, Zubicaray et al. used the same doses and schedules as in adults (G-CSF at a daily dose of 10 μg/kg for 5 days, and plerixafor 0.24 mg/kg, administered 6–11 h before apheresis, both injected subcutaneously) when mobilization was expected to be unsuccessful due to unfavourable donor/receptor weight ratio. Plerixafor was used 'upfront', not waiting for CD34+ cell count. This strategy was more successful when compared with normal (10 μg/kg/24 h) or high (10 μg/kg/12 h) doses of G-CSF, especially when ≥5 $\times$ 10^6 CD 34+ cells/kg of recipient weight were collected [29].

After reviewing existing studies, it seems reasonable administering subcutaneous plerixafor after G-CSF mobilization failure at a dose of 0.24–0.32 mg/kg and starting leukocytapheresis as early as 4 h and not longer than 12 h after plerixafor administration (Figure 1). Thus, a CD34+ cell count can be performed in the morning, administering plerixafor if the count is poor and performing an apheresis session in the afternoon of the same day. Our group found that in autologous donors, CD34+ cell collection efficiency of same-day processes was higher when compared with next-day apheresis [58]. A Dutch group has shown a good correlation between data obtained with a fully automated haematology analyser and CD34+ cell count by flow cytometry. The haematology analyser detected CD34+ cells based on cell

TABLE 1 Mobilization data

First author [ref]	Healthy related donors			Mobilization strategy		CD34+ cells in peripheral blood			Timing[a] (h)	CD34+ cells collected (×10^6/kg recipient)		
	n	Gender (M/F)	Age (years)	G-CSF (μg/kg/day)	Plerixafor (mg/kg)	After G-CSF (/μl)	After plerixafor (/μl)	Increase		Without plerixafor	With plerixafor	Total
Devine [22]	25	20 M/25 F	24–60	No	0.24	No	16 (4–54)	No	4	No	2.9 (1.2–6.3)	2.9 (1.2–6.3)
Hauge [23]	6	All F	44 (35–55)	10–16 (3–4 days)	0.24	19 (10–41)	96 (19–157)	3.2 (1.6–12.8)	10	1.4 (0.4–3.4)	7.0 (0.9–13.3)	10.2 (2.3–13.3)
Gattillo [24]	10	5 M/5 F	57 (37–73)	10 (4 days)	0.35 (0.24–0.47)	27 (11–34)	41 (17–147)	2.8 (1.1–4.3)	9–11	1.2 (1.1–3.9)	4.2 (1.3–8.5)	5.9 (1.8–9.5)
Schroeder [25]	50	NR[b]	NR[b] (18–70)	No	0.08–0.48 (SC and IV)	No	23 (4–157)	No	4	No	2.9 (2.0–9.7)	2.9 (2.0–9.7)
Teipel [26]	35	NR[b]	NR[b]	7.5–10 (4 days)	0.24	NR[b]	NR[b]	NR[b]	10	1.0 (0.5–1.9) [c]	2.2 (0.9–3.8)[c]	NR[b]
De Greef [27]	23	16 M/7F	47 (24–60)	No	0.32	No	26 (9–71)	No	10 (8–11)	No	3.3 (1.9–6.5)	3.9 (1.9–6.5)
Chen [28]	64	41 M/23F	56 (range: 18–65)	No	0.24	No	19 (range: 1.7–52)	No	4	No	2.8 (0.3–9.6)	4.7 (0.9–9.6)
Zubicaray [29]	9	NR[b]	3 (range: 1–17)	10 (4 days)	0.24	NR[b]	208	Yes	6–11	NR[b]	11.42 (5–17.85)	NR[b]
Hölig [30]	37	17 M/20F	34 (IQR: 26–48)	15 (4 days)	0.24	15 (IQR: 12–18)	44 (IQR: 38–61)	Yes	12	1.31 (IQR: 0.8–1.65)	2.8 (IQR: 2.26–4.69)	5.16 (IQR: 3.06–6.10)
Kharya[d] [31]	25	10 M/15F	35 (9–51)	10 (5 days)	0.24	NR[b]	NR[b]	NR[b]	10–12	NR[b]	NR[b]	10
Cid [32]	30	9 M/21F	51 (IQR: 34–59)	10–16 (IQR: 4–6 days)	0.24	17 (IQR: 11–27)	56 (IQR: 39–81)	3.3 (IQR: 2.8–4.8)	11 (IQR: 10–12)	1.6 (IQR: 0.9–2.5)	5.0 (IQR: 3.5–5.8)	6.1 (IQR: 4.8–7.3)

Note: Summary of studies where recipients received haematopoietic progenitor cells obtained from healthy related donors mobilized with plerixafor. Quantitative variables are given as median (range).

[a]Time (h) from plerixafor administration to leukocytapheresis start.

[b]NR: Not reported.

[c]Total CD34+ cells collected (x10^8).

[d]Haploidentical transplantation for sickle cell disease. All donors had sickle cell trait.

size, internal complexity and fluorescence of immature cells. This could provide a faster way to evaluate mobilization, further streamlining the process [59].

RESULTS IN MOBILIZATION AND HCT OUTCOMES

A successful mobilization cycle can be defined as one that collects the desired amount of CD34+ cells to perform a transplant in the defined time, ideally in a single apheresis session. A sufficient HPC dose is a significant predictive factor in the outcome of HCT. In general, a dose of ≥4.0 × 10^6 CD34+ cells/kg of recipients' body weight is usually considered optimal for allogeneic HCT, while doses between 2 and 4 × 10^6 CD34+ cells/kg of recipient's body weight are considered suitable for transplantation, albeit with inferior survival [60]. It should be also borne in mind that high doses of CD34+ cells (>8.0 × 10^6/kg) in cases of allogeneic HLA-identical sibling transplantation are associated with increased mortality from chronic GVHD [61–63].

Table 1 summarizes mobilization data of HDs after receiving plerixafor with or without G-CSF. In summary, plerixafor achieved mobilization of CD34+ cells, and, in nearly all cases, enough CD34+ cells for HCT could be collected, either in an upfront or in a just in time context.

Table 2 shows main results of allogeneic HCT using CD34+ cells collected from HDs who received plerixafor. Briefly, when plerixafor was used as an adjuvant to G-CSF or as a sole mobilization agent, no significant differences could be found in terms of time to haematopoietic engraftment, acute and chronic GVHD or overall survival when compared with similar patients who received G-CSF grafts. However, experience with this kind of products is still very limited to reach conclusions.

WHICH ARE THE ADVERSE EFFECTS?

Plerixafor is usually well tolerated, with rare serious adverse effects. In autologous donors, common adverse effects are diarrhoea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness and vomiting. Thrombocytopaenia is also a known adverse effect of plerixafor [20, 21].

Most HDs referred mild, grade-1 toxicity similar to that described during autologous donation. The most commonly reported adverse effects were digestive (loose stools, diarrhoea, nausea, bloating and flatulence), light-headedness, diaphoresis, perioral paresthaesia and erythema at the injection site. When donors received a second

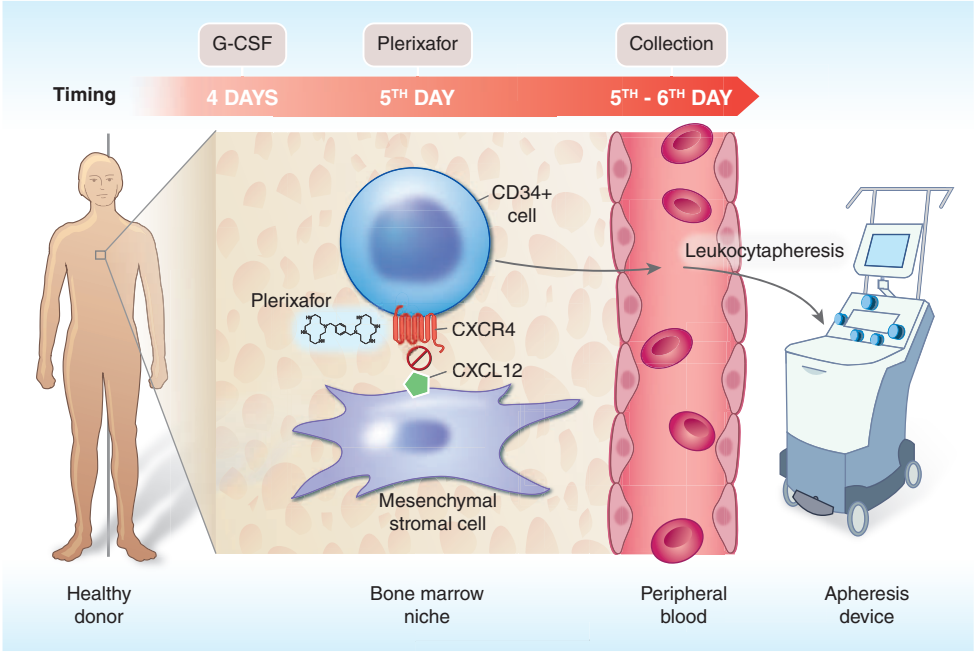


FIGURE 1 Schematic representation of CD34+ cell mobilization in healthy donors from bone marrow to peripheral blood using G-CSF and plerixafor. When the number of CD34+ cells in peripheral blood on the fifth day after starting mobilization with G-CSF and/or the predicted number of CD34+ cells collected are too low, the administration of subcutaneous plerixafor at a dose of 0.24–0.32 mg/kg blocks CXCR4 that is located on CD34+ cell membrane, and its union to mesenchymal stromal cells via CXCL12 is disrupted. Therefore, CD34+ cells migrate from bone marrow niche to peripheral blood. Leukocytapheresis can be started as early as 4 h and not longer than 12 h after plerixafor administration

TABLE 2 Haematopoietic cell transplantation (HCT) outcomes

First author [ref]	Patients			Diagnosis[a] (AL/Lym/other)	Conditioning regimen[b] (M/NM)	CD34+ cells ($\times 10^6$ /kg)	Engraftment (days after HCT)		Outcomes[c]		
	n	Gender	Age (years)				Neutrophils (>0.5 $\times$ 10^9/L)	Platelets (>20 $\times$ 10^9/L)	aGVHD	cGVHD	OS
Devine [22]	25	10 M/15F	52 (31–65)	14/6/5	M	2.9 (1.2–6.3)	10 (8–13)	12 (8–32)	35% (95% CI: 16–64)	33% (95% CI: 16–68) at 1 year	73% (95% CI: 63–87) at 1 year
Hauge [23]	6	4 M/2F	42 (15–52)	1/0/5	No/M/NM	10.2 (2.3–13.3)	13 (9–22)	17 (13–32)	NR[d]	NR[d]	NR[d]
Gattillo [24]	10	2 M/8F	51 (27–65)	9/0/1	NM	5.9 (1.8–9.5)	17 (15–25)	18 (10–101)	3 patients	2 patients	NR[d]
Schroeder [25]	33	NR[d]	NR[d]	17/13/3	M/NM	2.9 (2.0–9.7)	14 (11–27)	25 (15–219)[e]	21%	35%	45% at 1 year
Teipel [26]	18	NR[d]	NR[d]	NR[d]	NR[d]	NR[d]	17 (11–119)	17 (13–414)	NR[d]	NR[d]	NR[d]
De Greef [27]	23	17 M/6F	50 (21–64)	13/6/4	M/NM	3.9 (1.9–6.5)	17 (0–27)	13 (0–23)[e]	26%	30%	72% (49–87)
Chen [28]	63	40 M/23F	55 (19–69)	33/12/18	M/NM	4.7 (0.9–9.6)	13–15[f]	18–19[f]	M: 53% (95% CI: 35–71) NM: 18% (95% CI: 7–33)	M: 52% (95% CI: 33–70) NM: 39% (95% CI: 23–57)	M: 63% (95% CI: 46–79) NM: 70% (95% CI: 53–84)
Zubicaray [29]	9	NR[d]	3 (1–17)	NR[d]	NR[d]	11.42	NR[d]	NR[d]	NR[d]	NR[d]	NR[d]
Hölig [30]	37	17 M/20F	34 (IQR: 26–48)	NR[d]	NR[d]	3.74 (IQR: 2.26–4.69)	18 (IQR: 16–20)	17 (IQR: 13–30)	Grade 2–4:25% (95% CI, 13–50%)	N.R.	Survival probability at 2 years: 66% (95% CI, 49–89%)
Kharya[g] [31]	25	16 M/9F	35 (9–51)	Sickle cell disease	NM	10	13 (IQR: 12–15)	13 (IQR: 12–15)	Grade 2–4:20	Grade 1–2: 13	88
Cid [32]	25	NR[d]	NR[d]	NR[d]	NR[d]	6.1 (IQR: 4.8–7.3)	18 (IQR: 16–21)	19 (IQR: 16–26)	NR[d]	NR[d]	NR[d]

Note: Summary of studies where recipients received haematopoietic progenitor cells obtained from healthy related donors mobilized with plerixafor. Quantitative variables are given as median (range).

[a]Diagnosis. AL: acute leukaemia, Lym: lymphoma, MM: multiple myeloma.

[b]Conditioning regimen: M: myeloablative, NM: non-myeloablative.

[c]Outcomes: aGVHD: acute graft-versus-host disease, cGVHD: chronic graft-versus-host disease, OS: overall survival.

[d]NR: Not reported.

[e]Platelet engraftment: >50 $\times$ 10^9/L.

[f]Only median was reported.

[g]Haploidentical transplantation for sickle cell disease. All donors had sickle cell trait.

dose of plerixafor, there was no evidence of cumulative or more severe toxicity [22–25, 27, 28]. Splenic rupture has also been reported in HDs when plerixafor was used with G-CSF [20]. Hölig et al. recently reported the use of plerixafor as salvage treatment for poor mobilization. The application of plerixafor on the second apheresis day changed the side effects from pain, associated with G-CSF, towards gastrointestinal symptoms (diarrhoea, nausea, bloating) and local itching, and two patients had grade-3 thrombocytopaenia. However, 86% of donors agreed to receive plerixafor again if needed, and only one would have refused [30].

In healthy children, adverse effects were similar to those found in adults and were difficult to separate from those induced by G-CSF [64]. Among nine cases reported by Zubicaray et al. receiving plerixafor and G-CSF, adverse events related to the mobilization regime were found in five of nine donors (55.5%), each of them presenting one or several simultaneous adverse events: three of them reported nausea, two reported bone pain, one case of a low-grade fever, another of myalgia and another one suffered abdominal discomfort [29].

There are insufficient data on the use of plerixafor in pregnant women, but its pharmacodynamic mechanism of action suggests that plerixafor may cause birth defects when administered during pregnancy. Studies in animals have shown teratogenicity. Women of childbearing age should take contraceptive measures while taking plerixafor [20].

Patients with sickle cell disease (SCD) cannot receive G-CSF due to the risk of inducing vaso-occlusive and pain crises, but this has not been observed in patients with sickle trait. This is also the case for HDs who are heterozygous for sickle cell. In patients with SCD, plerixafor alone can be used for HPC mobilization, albeit with care. In a study promoted by the NIH, 15 patients were given plerixafor as a sole agent for CD34+ cell mobilization. Although a prophylactic RBC exchange had been performed, seven grade-3 adverse events (two non-pain-related and five pain-related) and one grade-4 haemolysis occurred, resolved with symptomatic treatment. Eleven participants experienced pain (grade 1–4), with three participants accounting for the five pain-related grade 3–4 AE [65]. In a recent paper, a group of 25 patients with SCD underwent HCT as a curative option for their disease. All donors, all of whom had a sickle cell trait, received G-CSF (10 μg/kg/24 h for 5 days) with upfront plerixafor (0.24 mg/kg, administered 10–12 h before apheresis). All donors suffered bone pain, but otherwise, the procedure was safe and all procedures achieved enough CD34+ cells for HCT. Interestingly, an autologous backup (>5 × 10^6 CD34+ cells/kg) was also collected from all patients using 2–3 days of G-CSF (5–10 μg/kg) with plerixafor (0.24 mg/kg 4–6 h prior to harvest). Only one patient needed exchange transfusion due to occlusive crisis [31].

CELL COMPOSITION OF THE GRAFT AFTER PLERIXAFOR USE

Outcomes of allogeneic HCTs performed using CD34+ cells collected from HDs mobilized with plerixafor may be better than allogeneic HCTs performed with CD34+ cells collected after mobilization of HDs without plerixafor. This can be deduced from two phase I/II studies where authors observed that the median time of neutrophil and platelet engraftments was similar in plerixafor- and G-CSF-mobilized HCTs, but the rates of acute and chronic GVHD could be lower when using plerixafor-mobilized HCTs, either using plerixafor after G-CSF mobilization failure or after using plerixafor alone [22, 25].

A possible explanation for the previous observations could be the result of different mobilization capabilities of G-CSF and plerixafor, resulting in a different transplant product when plerixafor is used alone or associated with G-CSF. However, much of our knowledge derives from autologous studies, where chemotherapy and the patient's disease can alter cellular populations in the BM or lymphoid organs [60, 66]. This can be particularly important when the mobilization regime includes chemotherapy.

First, regarding the characteristics of CD34+ cells mobilized with plerixafor, data on HDs are scarcer than in autologous donors, and one should also bear in mind that plerixafor is frequently given to bad mobilizers after receiving G-CSF. However, when using plerixafor alone, Schroeder et al. found that plerixafor mobilized more $CD34^{dim}$cells, with more CD34+ CD45RA+ CD123+ cells than when using G-CSF. They also found that HPCs expressed increased CXCR4 that could promote faster homing and more stable graft [25]. Moreover, De Greef et al., after using plerixafor alone, found a higher proportion of an immature CD34+ cell subset (lin-/CD34+/$CD38^{low}$/CD45RA-/CD90+) (31% vs. 15% with G-CSF) and 55% more committed progenitors (CD34+/CD45RA-/CD90+) versus 77% with G-CSF [27]. Although limited data are available, it seems that more immature CD34+ cells were mobilized with plerixafor alone in HDs.

Second, other cell populations collected after mobilization of HDs with plerixafor alone showed differences when compared with mobilization with G-CSF alone. Devine et al. obtained grafts with more CD3+ and CD4+ lymphocytes in HDs mobilized with plerixafor [22]. Teipel et al. compared cellular products in G-CSF mobilized HDs before and after additional plerixafor. Plerixafor induced a significant increase of total nucleated cells, including CD19+ B cells, CD4+ and CD8+ T cells and CD34+ haematopoietic stem and progenitor cells. More primitive CD34+ cells were also mobilized (CD34+CD38-CD133+), with a higher engraftment power. The number of plasmacytoid dendritic cells (DCs) also increased significantly, a cellular subset known for its tolerogenic properties inducing regulatory T cells (Treg), while no changes were detected for myeloid DCs. Absolute count of Tregs increased, especially naïve CD45RA+ Tregs, an unprimed or naïve Treg cell population. This population could compensate the risk of chronic GVHD posed by the increased number of CD8+ T cells mobilized after adding plerixafor. Higher numbers of myeloid-derived suppressor cells were detected after adding plerixafor [26]. Schroeder et al., using plerixafor alone, showed that although CD34+CD45RA- showed a genetic pathway analysis compatible with GVHD, this finding did not result in an increased incidence of GVHD, indeed the incidence was lower. They also found that plerixafor mobilized a subset of CD34dimCD45RA+CD123++ pro-DC2 (progenitors of pre-DC 2), that produced interferon-α (IFN-α) similar to mature p-DC2. This could explain the low incidence of CMV viremia in their patients, perhaps due to reduced incidence of chronic GVHD and immunosuppression [25]. Chen et al. detected that products obtained

after mobilization with plerixafor alone were not only richer in CD3+, CD4+ and CD8+ cells but also richer in $CD8^{hi}$ subset of T cells. This cellular subset has been associated with improved survival due to less relapse without increased GVHD and obtained from younger donors. Importantly, this was achieved although donors were older than had been previously published [28]. De Greef et al. found that after mobilization with plerixafor alone, CD3+ cells were increased compared to G-CSF, but CD4/CD8 ratios, frequency of Th2, Th17, NK cells (CD3-/CD16+/CR56+) and regulatory T cells were comparable, and CD19+ B cells were twice as high [27]. Couban et al. remarked that plerixafor alone mobilized earlier CD34+ progenitor cells, but their number did not correlate with their engraftment capability. They also found that plerixafor alone mobilized $CD56^{bright}$ NK_{reg} more efficiently than G-CSF or G-CSF with plerixafor [5]. This could be explained because NK and NKT populations express CXCR3 and CXCR4, essential for their homing into secondary lymphoid organs. This could be a key finding, as products containing $CD56^{bright}$ NK_{reg} and pre-DC cells may result in a lower rate of chronic GVHD, similar to recipients of BM grafts [67]. In summary, plerixafor mobilized immature lymphoid populations that appeared to yield a more tolerant product.

PLERIXAFOR AS A SOLE MOBILIZATION AGENT

Plerixafor can be used as a sole mobilization agent when G-CSF cannot be used due to allergy, previous adverse events or in the case of patients with SCD [22, 25, 27, 28, 65].

In general, donor toxicity after administering plerixafor alone was reduced when compared with G-CSF mobilized donors, with significantly less grade-2 to -4 toxicity. The amount of CD34+ cells in PB increased up to eight-fold in trials, but it was lower when compared with G-CSF. As a result, mobilizations with plerixafor yielded fewer CD34+ cells than G-CSF, and many donors failed to collect the desired number of cells in a single apheresis session. In the largest trial, 98% of 64 donors achieved the desired CD34+ cell dose (>4 $\times$ 10^6/kg), but only 27% achieved the ideal dose in one apheresis session. However, the overall failure rate after two apheresis sessions was low (2%–8%), and nearly all donations could be used for transplant [28]. Failure rates, as defined by authors, ranged from 33% to 50% in other studies [22, 25, 27]. On the other hand, engraftment, immune reconstitution, CMV reactivation or chronic GVHD did not differ significantly when compared with HCT performed with CD34+ cells mobilized with G-CSF.

Notwithstanding the evident benefits of this approach, mobilization of CD34+ cells from BM to PB in HDs with G-CSF remains the standard. However, the results of prospective, randomized controlled trials are keenly awaited to support this new approach.

RESEARCH INITIATIVES

A need to improve HPC collection exists as more clinical conditions can be successfully treated with HCTs and more patients gain access to treatment, especially from alternative sources (haploidentic and non-related donors who are under-represented in donor registries [68] or in resource-strapped countries [31]). Moreover, mobilization and collection of CD34+ cells form PB remain a complicated chore, even if enough HPCs can be collected. Multiple injections over several days can disrupt a donor's lifestyle. Mobilization protocols with G-CSF have side effects including bone pain, nausea, headache and fatigue, both in auto- and allogeneic donors. More severe side effects, like fatal spleen rupture, have also been reported [69]. The need of a venous central catheter to gain an adequate venous access adds to the inconvenience. In expert hands, about 10% of adult HDs need a central vein catheter, but at least 40% of small children can need a femoral catheter, in some cases for several days [48]. All these problems become more relevant for non-related HDs.

From the experiences mentioned above, some authors [5] suggested using plerixafor alone as the optimal agent to mobilize CD34+ cells for allogeneic HCT, as it can produce a HPC product with good engraftment characteristics, less adverse events than G-CSF mobilization, possibly equal or less chronic GVHD and more convenience for the donor and hospital as fewer doses are needed when compared with mobilization with G-CSF + plerixafor or just G-CSF alone [70, 71]. The high cost of plerixafor hinders its widespread use. However, Watts et al. found that in the autologous context, plerixafor was not associated with increased cost when mobilizing with G-CSF, due to the reduction in the number of aphesis and G-CSF doses [72]. Andritsos et al. reported 241 myeloma patients mobilized with upfront plerixafor, where enhanced collection efficiency, with most patients achieving collection in 1 day, resulted in cost savings [73]. Such studies have not been performed for HDs yet.

As a result, the search for the ideal mobilizing agent of CD34+ cells is ongoing. Inhibitors of integrin VLA4 (BIO5192), synthetic polysaccharides, proteasome inhibitors, inhibitors of prostaglandin E2 signalling (meloxicam) and interference with neuropeptide Y receptors have been tested, alone or associated with G-CSF or plerixafor [36]. Some of the most promising studies have targeted CXCR2. Hoggat et al. showed a fast and effective CD34+ cell mobilization when using plerixafor associated with a truncated form of the chemokine GRO-β [33]. Truncated GRO-β binds the CXCR2 receptor with greater potency than full GRO-β. Their experiments showed that while plerixafor acts by inhibiting CXCR4 directly on stem and progenitor cells, GRO-β along with plerixafor causes mobilization by targeting CXCR4 on neutrophils. In healthy volunteers, doses of GRO-β ≥ 60 μg/kg were capable to mobilize HPCs with apparently few adverse effects but not enough for clinical collection. The maximum tolerated dose was 200 μg/kg, and the main adverse effect was acute back pain that affected nearly all subjects. Applied to mice models, the association of GRO-β and plerixafor produced a peak mobilization 15 min after administration and achieved the collection of enough CD34+ cells for HCT. While each agent individually mobilized less than standard G-CSF regimes, the combination produced significantly greater results, partly explained by enhanced BM permeability. In this model, the transplant resulted a robust and stable haematopoietic engraftment, with quicker haematopoietic recovery, with greater

donor chimerism and which was more competitive than G-CSF mobilized grafts co-transplants. Indeed, GRO-β-plerixafor mobilized cells showed signatures in their transcriptome that resembled foetal liver HPCs. If these results could be replicated in humans, it could reduce the barriers to donation, especially for non-related donors.

An interesting proposal is using plerixafor to collect lymphoid cells for post-transplant immunotherapy or even for CAR-T cell production because plerixafor increased lymphoid populations in the transplant product [26].

Finally, plerixafor is under study as part of chemotherapy treatments for malignant diseases (mesothelioma, pancreatic cancer, hepatocellular carcinoma, ovarian cancer), as well as for a rare syndrome consisting of warts, hypogammaglobulinaemia, infections and myelokathexis (WHIM syndrome) [74] and autoimmune conditions (rheumatoid arthritis, systemic erythematous lupus) where the CXCR4 axis is involved [35].

CONCLUSIONS

Plerixafor is an effective and safe drug to overcome mobilization and collection failure of CD34+ cells after G-CSF administration in HDs. Plerixafor may also be used as a sole mobilization agent, with fewer side effects and more convenience than G-CSF mobilization protocols, but usually needing more than one apheresis session and probably would benefit from the assistance of other mobilizing drugs, still under research. However, more data and long-term follow-up are necessary to definitively establish the role of plerixafor in healthy allogeneic donors.

ACKNOWLEDGEMENTS

I.R. and C.C. designed the study, performed the literature review, wrote the first draft of the manuscript, and contributed in writing the final version of the manuscript. J.C. desgined the study, performed the literature review, revised the content of the drafts, and contributed in writing the final version of the manuscript. M.L. designed the study, revised the content of the drafts, contributed in writing the final version of the manuscript, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to VOX SANGUINIS.

ORCID

Iñigo Romon https://orcid.org/0000-0003-2428-4469
Carlos Castillo https://orcid.org/0000-0003-3766-8621
Joan Cid https://orcid.org/0000-0001-5445-4508
Miquel Lozano https://orcid.org/0000-0003-2593-833X

REFERENCES

1. Passweg JR, Baldomero H, Chabannon C, Basak GW, Corbacioglu S, Duarte R, et al. The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-T's come into focus. Bone Marrow Transplant. 2020;55:1604–13.
2. WMDA. WMDA Global Trends Report 2019. 2019. https://wmda.info/wp-content/uploads/2020/07/20201307-GTR-2019-Summary-slides.pdf. Accessed 24 Jan 2021.
3. Holtick U, Albrecht M, Chemnitz JM, Theurich S, Shimabukuro-Vornhagen A, Skoetz N, et al. Comparison of bone marrow versus peripheral blood allogeneic hematopoietic stem cell transplantation for hematological malignancies in adults - a systematic review and meta-analysis. Crit Rev Oncol Hematol. 2015;94:179–88.
4. Kollman C, Spellman SR, Zhang MJ, Hassebroek A, Anasetti C, Antin JH, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;127:260–7.
5. Couban S, Wong PC, Schultz KR. The case for plerixafor to replace filgrastim as the optimal agent to mobilize peripheral blood donors for allogeneic hematopoietic cell transplantation. Exp Hematol. 2019;70:1–9.
6. Spitzer TR, Kim SE, Cohen R, Mathews R, Li S, McAfee SL, et al. Declining bone marrow harvest quality over 24 years: a single institution experience. Bone Marrow Transplant. 2020;56:983–5.
7. Bredeson C, Leger C, Couban S, Simpson D, Huebsch L, Walker I, et al. An evaluation of the donor experience in the Canadian multicenter randomized trial of bone marrow versus peripheral blood allografting. Biol Blood Marrow Transplant. 2004;10:405–14.
8. Couzin C, Manceau S, Diana JS, Joseph L, Magnani A, Magrin E, et al. Vascular access for optimal hematopoietic stem cell collection. J Clin Apher. 2020;36:12–9.
9. Dogu MH, Kaya AH, Berber I, Sari İ, Tekgündüz E, Erkurt MA, et al. Does the preference of peripheral versus central venous access in peripheral blood stem cell collection/yield change stem cell kinetics in autologous stem cell transplantation? Transfus Apher Sci. 2016;54:76–9.
10. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. Cell Stem Cell. 2012;10:120–36.
11. Mohty M, Ho AD. In and out of the niche: perspectives in mobilization of hematopoietic stem cells. Exp Hematol. 2011;39:723–9.
12. Schmitz N, Dreger P. Peripheral blood hematopoietic cells for allogeneic transplantation. In: Forman SJ, Negrin RS, Antin JH, Appelbaum FR, editors. Thomas' hematopoietic cell transplantation. Chichester, England: Wiley Blackwell; 2016. p. 460–8.
13. Giralt S, Costa L, Schriber J, DiPersio J, Maziarz R, McCarty J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. Biol Blood Marrow Transplant. 2014;20:295–308.
14. DiPersio JF, Micallef IN, Stiff PJ, Bolwell BJ, Maziarz RT, Jacobsen E, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. J Clin Oncol. 2009;27:4767–73.
15. DiPersio JF, Stadtmauer EA, Nademanee A, Micallef INM, Stiff PJ, Kaufman JL, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. Blood. 2009;113:5720–6.
16. Beelen DW, Ottinger H, Kolbe K, Pönisch W, Sayer HG, Knauf W, et al. Filgrastim mobilization and collection of allogeneic blood progenitor cells from adult family donors: first interim report of a prospective German multicenter study. Ann Hematol. 2002;81:701–9.
17. Teipel R, Schetelig J, Kramer M, Schmidt H, Schmidt AH, Thiede C, et al. Prediction of hematopoietic stem cell yield after mobilization

with granulocyte-colony-stimulating factor in healthy unrelated donors. Transfusion. 2015;55:2855–63.
18. Ings SJ, Balsa C, Leverett D, Mackinnon S, Linch DC, Watts MJ. Peripheral blood stem cell yield in 400 normal donors mobilised with granulocyte colony-stimulating factor (G-CSF): impact of age, sex, donor weight and type of G-CSF used. Br J Haematol. 2006;134: 517–25.
19. Truong TH, Prokopishyn NL, Luu H, Guilcher GMT, Lewis VA. Predictive factors for successful peripheral blood stem cell mobilization and collection in children. J Clin Apher. 2019;34:598–606.
20. EMA. Plerixafor. ema.europa.eu, European Union, 2019. https://www.ema.europa.eu/en/medicines/human/EPAR/mozobil. Accessed 7 Sept 2019.
21. FDA. Mozobil drug approval package. 2008. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022311s000TOC.cfm. Accessed 24 Jan 2021.
22. Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. Blood. 2008;112:990–8.
23. Hauge AW, Haastrup EK, Sengelov H, Minulescu L, Dickmeiss E, Fischer-Nielsen A. Addition of plerixafor for CD34+ cell mobilization in six healthy stem cell donors ensured satisfactory grafts for transplantation. Transfusion. 2014;54:1055–8.
24. Gattillo S, Marktel S, Rizzo L, Malato S, Malabarba L, Coppola M, et al. Plerixafor on demand in ten healthy family donors as a rescue strategy to achieve an adequate graft for stem cell transplantation. Transfusion. 2015;55:1993–2000.
25. Schroeder MA, Rettig MP, Lopez S, Christ S, Fiala M, Eades W, et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. Blood. 2017;129: 2680–92.
26. Teipel R, Oelschlägel U, Wetzko K, Schmiedgen M, Kramer M, Rücker-Braun E, et al. Differences in cellular Composition of peripheral blood stem cell grafts from healthy stem cell donors mobilized with either granulocyte colony-stimulating factor (G-CSF) alone or G-CSF and Plerixafor. Biol Blood Marrow Transplant. 2018;24: 2171–7.
27. de Greef GE, Braakman E, van der Holt B, Janssen JJWM, Petersen E, Vucinic V, et al. The feasibility and efficacy of subcutaneous plerixafor for mobilization of peripheral blood stem cells in allogeneic HLA-identical sibling donors: results of the HOVON-107 study. Transfusion. 2019;59:316–24.
28. Chen YB, Le-Rademacher J, Brazauskas R, Kiefer DM, Hamadani M, DiPersio JF, et al. Plerixafor alone for the mobilization and transplantation of HLA-matched sibling donor hematopoietic stem cells. Blood Adv. 2019;3:875–83.
29. Zubicaray J, Galvez E, Sebastian E, Molina B, González-Vicent M, Castillo A, et al. Plerixafor-based mobilization in pediatric healthy donors with unfavorable donor/recipient body weight ratio resulted in a better CD34(+) collection yield: a retrospective analysis. J Clin Apher. 2020;36:78–86.
30. Holig K, Schmidt H, Hutter G, Kramer M, Teipel R, Heidrich K, et al. Salvage treatment with plerixafor in poor mobilizing allogeneic stem cell donors: results of a prospective phase II-trial. Bone Marrow Transplant. 2020;56:635–45.
31. Kharya G, Bakane A, Agarwal S, Rauthan A. Pre-transplant myeloid and immune suppression, upfront plerixafor mobilization and post-transplant cyclophosphamide: novel strategy for haploidentical transplant in sickle cell disease. Bone Marrow Transplant. 2020;56: 492–504.
32. Cid J, Monsalvo S, Castillo C, Pascual C, Moreno-Jiménez G, López-Parra M, et al. Addition of plerixafor to G-CSF in poor mobilizing healthy related donors overcame mobilization failure: an observational case series on behalf of the Grupo Espanol de Trasplante Hematopoyetico (GETH). Transfus Apher Sci. 2021;60: 103052.
33. Hoggatt J, Singh P, Tate TA, Chou B-K, Datari SR, Fukuda S, et al. Rapid mobilization reveals a highly engraftable hematopoietic stem cell. Cell. 2018;172:191–204.e10.
34. Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. Antimicrob Agents Chemother. 2000;44:1667–73.
35. Wang J, Tannous BA, Poznansky MC, Chen H. CXCR4 antagonist AMD3100 (plerixafor): from an impurity to a therapeutic agent. Pharmacol Res. 2020;159:105010.
36. Pelus LM, Broxmeyer HE. Peripheral blood stem cell mobilization; a look ahead. Curr Stem Cell Rep. 2018;4:273–81.
37. Guyon A. CXCL12 chemokine and its receptors as major players in the interactions between immune and nervous systems. Front Cell Neurosci. 2014;8:65.
38. Nagasawa T. CXCL12/SDF-1 and CXCR4. Front Immunol. 2015; 6:301.
39. De Clercq E. AMD3100/CXCR4 inhibitor. Front Immunol. 2015; 6:276.
40. Liles WC, Broxmeyer HE, Rodger E, Wood B, Hübel K, Cooper S, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood. 2003;102:2728–30.
41. Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, et al. Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist. Transfusion. 2005;45:295–300.
42. Allen ES, Conry-Cantilena C. Mobilization and collection of cells in the hematologic compartment for cellular therapies: stem cell collection with G-CSF/plerixafor, collecting lymphocytes/monocytes. Semin Hematol. 2019;56:248–56.
43. Douglas KW, Gilleece M, Hayden P, Hunter H, Johnson PRE, Kallmeyer C, et al. UKconsensus statement on the use of plerixafor to facilitate autologous peripheral blood stem cell collection to support high-dose chemoradiotherapy for patients with malignancy. J Clin Apher. 2018;33:46–59.
44. Kriegsmann K, Wuchter P. Mobilization and collection of peripheral blood stem cells in adults: focus on timing and benchmarking. Methods Mol Biol. 2017;2019:41–58.
45. Hübel K. Mobilization and collection of HSC. In: Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT handbook hematopoietic stem cell transplantation and cellular therapies. Cham, Switzerland: Springer Nature Switzerland AG; 2019. p. 117–22.
46. Kimura S, Ohkawara H, Minakawa K, Fukatsu M, Mori H, Takahashi H, et al. Optimal timing of apheresis for the efficient mobilization of peripheral blood progenitor cells recruited by high-dose granulocyte colony-stimulating factor in healthy donors. Transfus Apher Sci. 2020;59:102737.
47. Kong JH, Hu Y, Shim H, Lee E, Lee H, Eom HS, et al. Analysis of factors associated with successful allogeneic peripheral blood stem cell collection in healthy donors. Transfus Apher Sci. 2020;59:102679.
48. Benakli M, Ahmed Nacer R, Mehdid F, Belhadj R, Rahmoune N, Baazizi M, et al. Peripheral blood stem cell mobilization and collection in pediatric healthy sibling donors weighing 20 kilograms or less; algerian experience. Transfus Apher Sci. 2020;59:102987.
49. Suzuya H, Watanabe T, Nakagawa R, Watanabe H, Okamoto Y, Onishi T, et al. Factors associated with granulocyte colony-stimulating factor-induced peripheral blood stem cell yield in healthy donors. Vox Sang. 2005;89:229–35.
50. Leotta S, Poidomani M, Mauro E, Spadaro A, Marturano E, Milone G. AMD3100 for urgent PBSC mobilization and allogeneic

transplantation from a normal donor after failed marrow harvest. Bone Marrow Transplant. 2011;46:314–6.
51. Schriber J, Fauble V, Sproat LO, Briggs A. Plerixafor 'just in time' for stem cell mobilization in a normal donor. Bone Marrow Transplant. 2011;46:1026–7.
52. Eyre TA, King AJ, Peniket A, Rocha V, Collins GP, Pawson R. Partial engraftment following plerixafor rescue after failed sibling donor peripheral blood stem cell harvest. Transfusion. 2014;54:1231–4.
53. Neumann T, Kruger WH, Busemann C, Kiefer T, Dolken G. Successful mobilization of PBSCs in a healthy volunteer donor by addition of plerixafor after failure of mobilization with G-CSF alone. Bone Marrow Transplant. 2011;46:762–3.
54. Pantin J, Purev E, Tian X, Cook L, Donohue-Jerussi T, Cho E, et al. Effect of high-dose plerixafor on CD34+ cell mobilization in healthy stem cell donors: results of a randomized crossover trial. Haematologica. 2017;102:600–9.
55. Goren Sahin D, Arat M. Peripheral blood stem cell collection for allogeneic hematopoietic stem cell transplantation: practical implications after 200 consequent transplants. Transfus Apher Sci. 2017;56:800–3.
56. Hauser RG, Kwon RJ, Ryder A, Cheng C, Charifa A, Tormey C. Transfusion medicine equations made internet accessible. Transfus Med Rev. 2020;34:5–9.
57. Bartnik K, Maciejewska M, Farhan R, Urbanowska E, Król M, Król M, et al. Continuous mononuclear cell collection (cMNC) protocol impact on hematopoietic stem cell collections in donors with negative collection predictors. Transfus Apher Sci. 2018;57:401–5.
58. Cid J, Castillo C, Marin P, Carbassé G, Herrera D, Monfort N, et al. Increased collection efficiency of CD34+ cells after mobilization with preemptive use of plerixafor followed by leukocytapheresis on the same day. Transfusion. 2020;60:779–85.
59. Gromme M, Russcher H, Braakman E, Klinkspoor JH, Dobber JA, de Greef I, et al. Multicenter study to evaluate a new enumeration method for hematopoietic stem cell collection management. Transfusion. 2017;57:1949–55.
60. Valtola J, Silvennoinen R, Ropponen A, Siitonen T, Säily M, Sankelo M, et al. Blood graft composition and post-transplant recovery in myeloma patients mobilized with plerixafor: a prospective multicenter study. Leuk Lymphoma. 2019;60:453–61.
61. Mohty M, Bilger K, Jourdan E, Kuentz M, Michallet M, Bourhis JH, et al. Higher doses of CD34+ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. Leukemia. 2003;17:869–75.
62. Mielcarek M, Martin PJ, Heimfeld S, Storb R, Torok-Storb B. CD34 cell dose and chronic graft-versus-host disease after human leukocyte antigen-matched sibling hematopoietic stem cell transplantation. Leuk Lymphoma. 2004;45:27–34.
63. Urbano-Ispizua A, Carreras E, Marin P, Rovira M, Martínez C, Fernández-Avilés F, et al. Allogeneic transplantation of CD34(+) selected cells from peripheral blood from human leukocyte antigen-identical siblings: detrimental effect of a high number of donor CD34 (+) cells? Blood. 2001;98:2352–7.
64. Karres D, Ali S, van Hennik PB, Straus S, Josephson F, Thole G, et al. EMA recommendation for the pediatric indications of plerixafor (Mozobil) to enhance mobilization of hematopoietic stem cells for collection and subsequent autologous transplantation in children with lymphoma or malignant solid tumors. Oncologist. 2020;25:e976-e81.
65. Uchida N, Leonard A, Stroncek D, Panch SR, West K, Molloy E, et al. Safe and efficient peripheral blood stem cell collection in patients with sickle cell disease using plerixafor. Haematologica. 2020;105:e497.
66. Worel N, Frank N, Frech C, Fritsch G. Influence of plerixafor on the mobilization of CD34+ cell subpopulations and lymphocyte subtypes. Transfusion. 2017;57:2206–15.
67. Waller EK, Logan BR, Harris WA, Devine SM, Porter DL, Mineishi S, et al. Improved survival after transplantation of more donor plasmacytoid dendritic or naive T cells from unrelated-donor marrow grafts: results from BMTCTN 0201. J Clin Oncol. 2014;32:2365–72.
68. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014;371:339–48.
69. Benguerfi S, Thepault F, Lena H, Ricordel C. Spontaneous splenic rupture as a rare complication of G-CSF injection. BMJ Case Rep. 2018;2018.
70. Azar N, Ouzegdouh M, Choquet S, Leblond V. Impact of plerixafor (mozobil) on hospital efficiency: a single center experience. J Clin Apher. 2018;33:5–13.
71. Verlinden A, Van de Velde A, Verpooten GA, van Doorn KJ. Determining factors predictive of CD34+ cell collection efficiency in an effort to avoid extended and repeated apheresis sessions. J Clin Apher. 2013;28:404–10.
72. Watts NL, Marques MB, Peavey DB, Innis-Shelton R, Saad A, AD S, et al. Mobilization of hematopoietic progenitor cells for autologous transplantation using Pegfilgrastim and Plerixafor: efficacy and cost implications. Biol Blood Marrow Transplant. 2019;25:233–8.
73. Andritsos LA, Huang Y, Abraham I, Huff K, Scrape SR, Fan T, et al. Clinical and cost outcomes of pre-emptive plerixafor administration in patients with multiple myeloma undergoing stem cell mobilization. Leuk Res. 2019;85:106215.
74. McDermott DH, Pastrana DV, Calvo KR, Pittaluga S, Velez D, Cho E, et al. Plerixafor for the treatment of WHIM syndrome. N Engl J Med. 2019;380:163–70.

How to cite this article: Romon I, Castillo C, Cid J, Lozano M. Use of plerixafor to mobilize haematopoietic progenitor cells in healthy donors. Vox Sang. 2022;117:6–16.

Received: 16 December 2020 | Revised: 30 March 2021 | Accepted: 30 March 2021

DOI: 10.1111/vox.13126

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The impact of donor ferritin testing on blood availability in Canada

John T. Blake[1,2] | Sheila F. O'Brien[2,3] | Mindy Goldman[2,4]

[1]Industrial Engineering, Dalhousie University, Halifax, Nova Scotia, Canada

[2]Canadian Blood Services, Centre for Innovation, Ottawa, Ontario, Canada

[3]School of Epidemiology and Public Health, University of Ottawa, Ottawa, Ontario, Canada

[4]Department of Pathology & Laboratory Medicine, University of Ottawa, Ottawa, Ontario, Canada

Correspondence
John T. Blake, Industrial Engineering, Dalhousie University, Halifax, Nova Scotia, Canada.
Email: john.blake@dal.ca

Funding information
Canadian Blood Services, Grant/Award Number: Operating Grant; Canadian Network for Research and Innovation in Machining Technology, Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: RGPIN-2014-06278

Abstract

Background and Objectives: Iron depletion is a side effect of blood donation. Agencies have developed policies to test donors and to extend inter-donation intervals (IDIs) for individuals with low ferritin levels. Ferritin testing, however, has an impact on product availability due to longer IDIs and the effect of test results on donor behaviour. In this paper we apply a model to evaluate the impact of ferritin testing in the Canadian donor population on whole blood donations.

Materials and Methods: A discrete event simulation was adopted for the study. The model represents a population of individuals that donate blood, are tested for ferritin levels, and may exit the system. Data for the simulation was derived from operational data, donor research studies from Canadian Blood Services and previously published sources.

Results: Red cell collections will decline by at least 3.1% and could decline by as much as 19.2% after ferritin testing is put in place. Requirements for new donors could rise by as much as 36.0%.

Conclusion: The impact of ferritin testing on repeat donor behaviour, rather than extensions to the mandated inter-donation interval, is the largest factor influencing declines in whole blood donations. Because behaviour changes following the receipt of a low ferritin result, blood agencies must ensure that donors with low ferritin are motivated to modify their lifestyle and, when healthy, return to the donor pool.

KEYWORDS
anaemia, blood donation, simulation

INTRODUCTION

While blood transfusion recipient safety has received much attention over the years, less attention has been paid to the impact of phlebotomy on donors. Historically, blood donation was thought to be innocuous. Nevertheless, it is known that blood donation impacts donor iron stores, as measured by serum ferritin levels [1], and that low iron stores represent a risk for donors [2, 3], particularly women of childbearing age. Recent focus on donor health, including studies conducted at Canadian Blood Services [4], suggest that frequent donation negatively impacts donor iron levels and can lead to iron depletion or anaemia [5]. While donor qualification usually relies on haemoglobin levels, haemoglobin and ferritin are poorly correlated and haemoglobin is, at best, an imperfect measure of iron stores [6, 7]. Ferritin testing, in conjunction with haemoglobin testing, offers improved accuracy in assessing donor iron status [8] . One method for ensuring the health of donors is to monitor ferritin in repeat donors and provide information to individuals with low ferritin levels to manage anaemia [9]. As a point-of-care ferritin assay is not available, donors must be informed of test results some time after their blood donation. This lag poses challenges in communication and behaviour modification. Donors with low ferritin levels can be encouraged to

consult their family physician and consider iron supplementation. In addition, donors may be temporarily deferred from blood donation [10]. Donors typically express positive attitudes towards receiving a ferritin result from a blood service [11], and are willing to take action following a low ferritin test result [10, 12].

Since the 1980's, changes to haemoglobin levels and inter-donation interval have been suggested in the literature [2], along with recommendations for monitoring serum ferritin levels in frequent blood donors [7, 13] to reduce iron depletion. The RISE study suggested that iron depletion is frequent in blood donors and showed a strong correlation between donation frequency and iron depletion [5]. Accordingly, blood agencies have increased the inter-donation interval (IDI) for donors [7]. In 2016 Canadian Blood Services (CBS) increased the IDI for women from 8 to 12 weeks. Modelling conducted before the change, indicated both temporary and permanent losses in donations due to the increased IDI [14]; CBS was able, however, to recruit new donors and encourage more donations from its existing donor population to make up for the forecast decrease in product availability [12]. In the REDS-II study, linear regression models were used to represent haemoglobin levels, ferritin levels, and inter-donation interval for donors attending facilities in the study. Results from this modelling suggest a reduction in whole blood donations of 12% when IDI was increased from 56 days for all donors to 112 days for females and 84 days for males [15]. If donors were tested for ferritin, rather than just haemoglobin, as is the case now, it is expected that additional donors would be flagged as iron deficient or absent iron; a 2016 study by Goldman et al. showed a 10-fold increase in iron levels below 26 μg/L in male donors having completed six or more donations in the previous year, when compared to first time donors [4].

Since the majority of the whole blood units provided in Canada come from repeat donors, it is important to understand the impact of donor ferritin testing, extended inter-donation interval, and donor behaviour, following a low ferritin result, on blood product availability. In this study, simulation is used to assess the impact on whole blood collections of ferritin testing for repeat donors for those individuals found to have a ferritin level below 26 μg/L.

METHODS

Overview

A custom-built simulation model was constructed using Visual Basic for Applications via MS-Excel. Once built and verified, the model was used to run a set of experiments focusing on blood product availability following the implementation of donor ferritin testing. The experiments were designed to determine the impact of both regulatory change to inter-donation interval and the effects of ferritin testing on donor behaviour.

Simulation model

The simulation consists of a series of class-objects representing donors, the ferritin test process, and a donor schedule. See Appendix A for a detailed list of objects and their properties and methods that form the simulation. The simulation assumes a fixed cohort of donors: 10,000 in the runs discussed in this paper. Each donor is imbued with a gender, age, a donation status (first time vs. repeat), and a preferred donation frequency. Assignment of donor characteristics is based on draws from discrete empirical distributions derived from CBS operational data. See Table S1 for a detailed listing of this data. Once created, a donor is scheduled for an appointment; next appointment dates are determined as follows:

$$TTN_j = \mathrm{Max}\left(U(0.9,1.1)*365/F_j, IDI_j\right),$$

where

TTN_j is the time to the next donation for donor j.

U(0.9, 1.1) is a draw from a uniform distribution with parameters (0.9, 1.1). The Uniform (0.9, 1.1) scaling factor was determined empirically via experiments with the simulation and comparisons against annual collection rates.

F_j is the preferred annual frequency for donor j.

IDI_j is the minimum inter-donation interval for donor j, which is based on the donor's gender and current ferritin status (if known).

Thus, a donor's next donation date is the maximum of his/her desired return date or his/her minimum specified inter-donation interval. Donors are then entered onto the donor list for their assigned appointment day by adding TTN_j to the current simulated day. See Figure 1 for a flow chart detailing donor initialization in the simulation.

A simulation loop is then executed. See Figure 2. On each simulated day, the routine searches the donor list for individuals with appointments on that day. If the donor is scheduled to provide a donation, he/she is removed from "today's" donor list, a whole blood unit is collected, and the donor is booked for his/her next appointment. Please note that the simulation model assumes that donors have sufficient haemoglobin to donate at all instances when scheduled. The next donation date is selected in the same manner as the donor's first appointment. If the next donation happens in the following calendar year, the donor is assumed to have aged. See Figure 3. Donors with appointments in the next calendar year also decide if they will return or not; retention rates vary by donor gender and age, but more than 50% of all first-time donors are lost to attrition, while approximately 13% of repeat donors currently exit the system annually. The decision to exit the donor pool is based on a draw from an empirical discrete distribution that accounts for gender, age, and donation frequency. See Table S2 for detailed data. For every donor lost, another first-time donor is assumed to be recruited and to enter the simulation as described above. Thus, the donor population in the simulation always remains a constant size in the simulation, even though individual donors come and go.

If the donor is eligible, a ferritin test is conducted at a donation event. Ferritin tests occur every fifth donor donation and results are returned as either normal (≥26 μg/L), low (restricted erythropoiesis, 12–25 μg/L) or very low (below 12 μg/L, absent iron). Test outcomes in the simulation are derived from an empirical distribution specific to

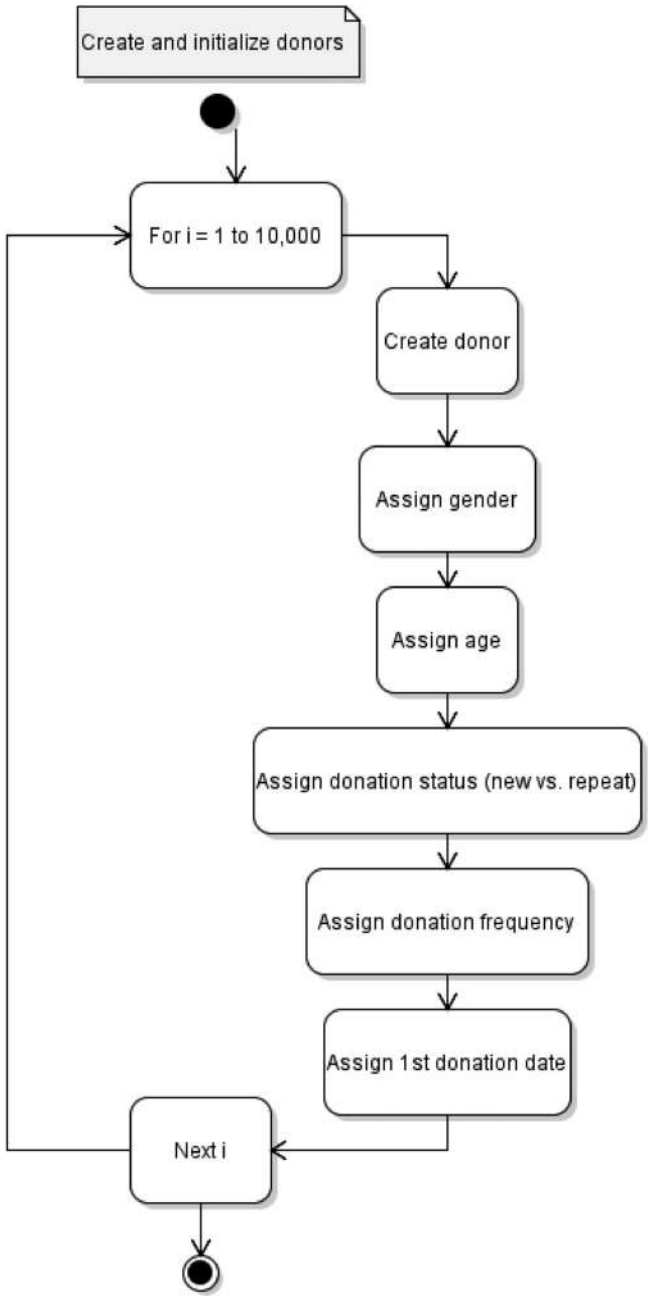


FIGURE 1 Process flow chart for simulation initiation

donor gender, age, and donation frequency; these empirical distributions were created from the CBS data set provided in Goldman et al. [4]. See Table S3 for detailed data. Donors that are tested and found to have a ferritin level below 26 μg/L have their next donation time altered to incorporate any extended inter-donation interval. See Figure 4 for a flow chart of the ferritin test process.

A recent Dutch study indicates that haemoglobin deferrals decrease significantly in donors returning from a ferritin deferral [7]. We assume, therefore, that any donor flagged as having low iron stores and deferred for a period greater than 6 months returns, after deferral, with a sufficient haemoglobin level to complete their next donation. Readers should note that, since ferritin testing is envisioned every fifth donation, the donor's ferritin level is unknown in the simulation (and in reality) until their next test, which may be several years in the future. Thus, all individuals completing a donation, regardless of ferritin level, are placed back on the donor list on the day of his/her next simulated appointment. However, in scenarios listed below, we also incorporate changes to donor behaviour that causes some repeat donors with low or absent ferritin levels to exit the simulation, to delay their return to donation (a temporary decrease in donation frequency), or to permanently decrease their donation frequency.

Readers may find a working copy of the Excel-based simulation model in File S1.

Data

Ferritin data for the model was taken from 12,595 CBS whole blood donors; this is the same source reported by Goldman et al. [4]. This data provides records of donor ferritin levels by gender, age, and number of donations in the previous year for a randomly selected sample of Canadian donors. Ferritin data was then combined with operational donor data for all whole blood donors in 2017 to build empirical distributions that determine the probability that a donor of a given gender, age, and donation history will have either a normal (≥26 μg/L), low (12–25 μg/L), or very low (below 12 μg/L) ferritin level. Data on donor behaviour following a low ferritin result, including donor retention, donor return interval, and revised donation frequency were taken from a follow up study conducted by Goldman et al. [10] at Canadian Blood Services in 2017.

Verification

Outputs closely related to input data were verified against the data used to populate the model. Donor gender, age distribution, and donation frequency were confirmed with z-tests or Chi-squared tests, as appropriate, and confirmed to be not statistically different from historical data. See Figure 5 for a comparison of age distribution for donors in the simulation against 2017 data; see Appendix B for detailed comparisons of donor gender and donation frequency. Because the model was shown to reproduce donor gender, age, and donation frequency accurately, it was assumed to be an acceptable representation of the Canadian Blood Services donor population for the purposes of evaluating ferritin testing policies.

Five scenarios were simulated in this study:

1. Scenario 1 (Current situation): For all male donors, the minimum inter-donation interval is 56 days, while for all female donors the minimum is 84 days; donors return to donate at the greater of their preferred inter-donation interval (an attribute of the donor) or the minimum IDI for their gender specified by the blood agency. In the simulation, individuals are assumed to have received ferritin tests every fifth donation. In reality, donors were not tested for ferritin in the current situation. However, we included simulated

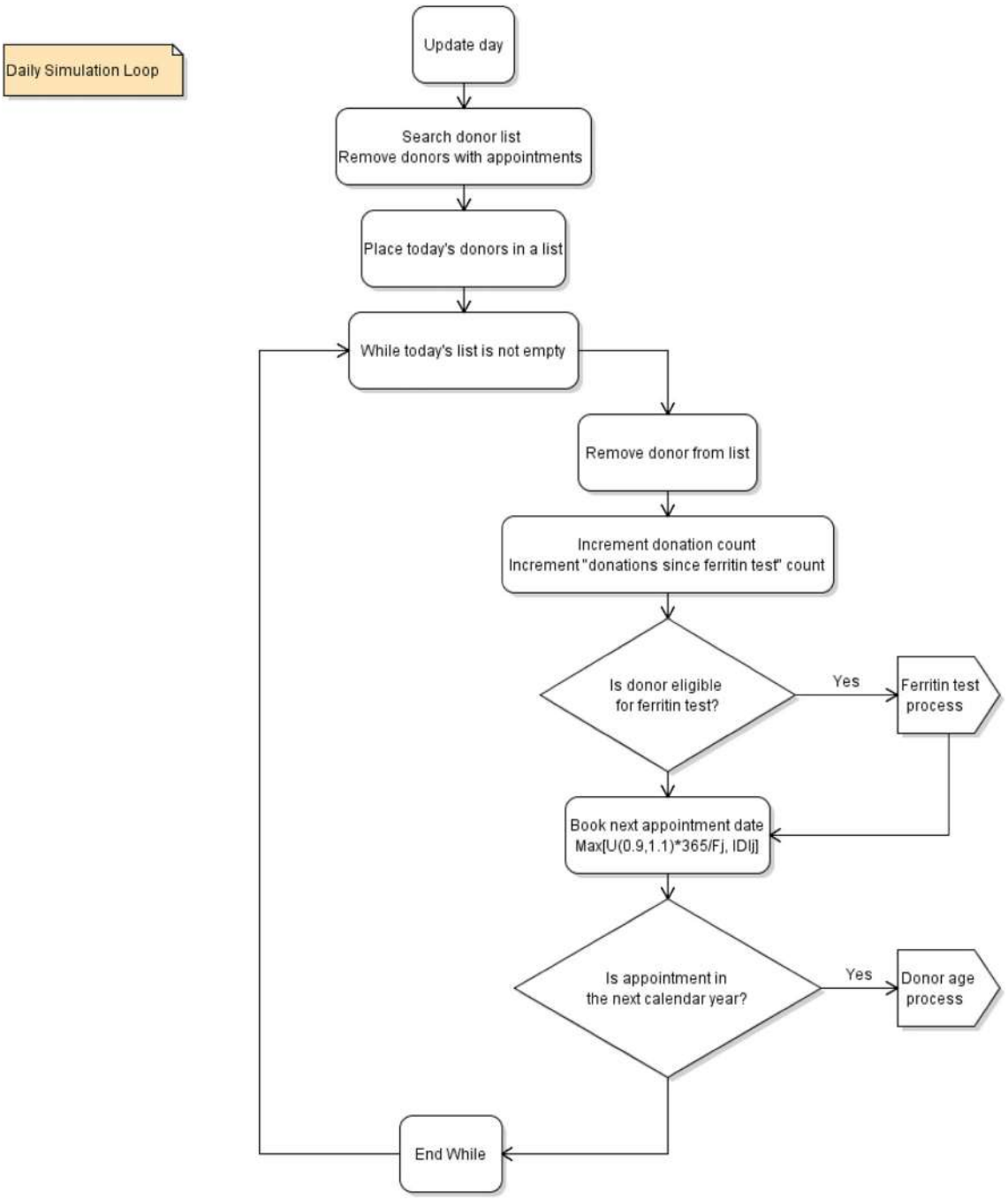


FIGURE 2 Process flow diagram detailing the daily simulation loop [Colour figure can be viewed at wileyonlinelibrary.com]

testing in this scenario to estimate the number of donation events where the donor would have low ferritin, absent of any intervention.

2. Scenario 2 (Extended IDI). Donors with acceptable ferritin levels (≥ 26 μg/L) can donate again after either 56 days, if male, or 84 days, if female. Donors with a low or very low ferritin level (<26 μg/L and <12 μg/L) are deferred for a six-month period. When donors return after an extended deferral time, they are assumed to return with a sufficient haemoglobin level to donate successfully. All donors are assumed to return to the donor pool and to resume their pre-test donation behaviour in this scenario.
3. Scenarios 3A-3C (Low ferritin influences donor behaviour): Donors with low or very low ferritin are advised to see their health care practitioner for follow-up. Donors may decide to pause or cease donation, either on their own accord, or with the advice of their health care practitioner. Donors may also alter their donation frequency, should they decide to return as a donor. In Scenario 3A, an extended IDI is assumed, along with a decrease in donor retention following a low ferritin test result. In Scenario 3B, extended

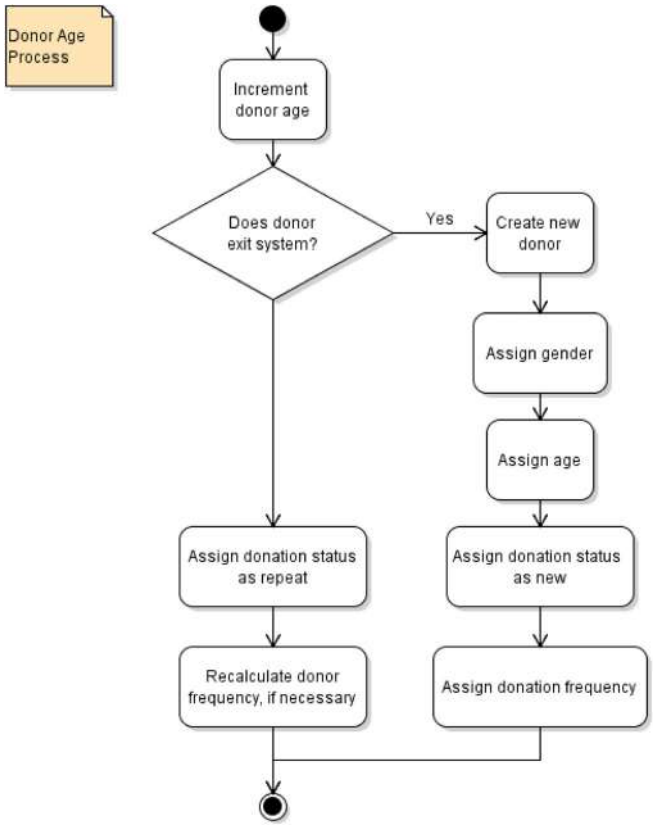


FIGURE 3 Process flow chart for donor aging [Colour figure can be viewed at wileyonlinelibrary.com]

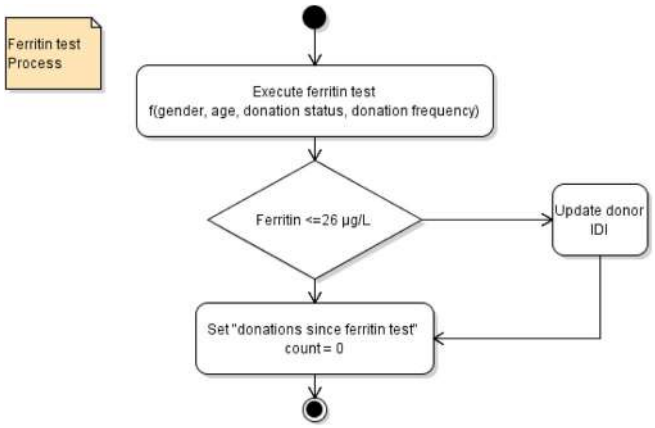


FIGURE 4 Process flow diagram for executing a ferritin test [Colour figure can be viewed at wileyonlinelibrary.com]

IDI and a reduced donation frequency is assumed following a low ferritin test. In Scenario 3C, and extended IDI, reduced donor retention, and a decreased donation frequency, are assumed following a low ferritin test.

To estimate behaviour change, anonymized donor data from 2014 through 2016 was obtained from Canadian Blood Services for participants in the ferritin testing study conducted by Goldman et al. [4]. Of the 12,595 individuals who were flagged as whole blood donors in that study 5,360 (42.5%) were notified that they had a low ferritin level. Two thousand thirty-three of the donors (38.0%) who were notified of a low ferritin level did not make a subsequent donation in the 24 months following their participation in the ferritin testing study. The retention rate (76.4%) for repeat male donors who received a low or very low ferritin test was similar to donors whose ferritin levels were above 26 µg/L (83.2%). However, the retention rate for repeat female donors with a low or very low ferritin test level was found to have declined from 77.4% to 62.0%. Moreover, donors receiving a low or very low ferritin result were found to delay their return to donation: 70% of males and 82% of females remain outside of the donor pool for longer than the minimum IDI of 6 months. Similar effects on donor behaviour have been reported elsewhere [7]. The receipt of a low ferritin test was observed in the data set to result in a decrease in donation frequency; an overall drop in donations of 1.3 instances per annum [N(1.5, 1.4) for males; N(1.0, 1.5) for females] was observed.

RESULTS

Scenario 2 (Extended IDI)

The expected number of donations per year and the change between the baseline and extended deferral scenario is presented in Table 1. The results suggest that a reduction in total donations of 24,363 (*95% CI Half Width: 1472*) can be expected for the extended IDI scenario, when compared with the baseline. The largest absolute loss will come from decreased donations from males, by 19,687 units (*95% CI Half Width: 438*). Overall, donations will decline by 3.1% (*95% CI Half Width: 0.2%*), with female donations expected to decline by 1.4% (*95% CI Half Width: 0.4%*) and donations from males to decrease by 4.3% (*95% CI Half Width: 0.1%*). Note that, by convention, we report the simulated average value and a 95% two-sided confidence half-width, based on an n of 30. A confidence interval, which is the mean value $+/-$ the half-width value, measures the error in the estimate of the true mean. See Reference [16, pp. 278–280] for calculation details, examples, and further background. Readers should also note that the simulation assumes a donor pool of 10,000 individuals; results reported, however, are scaled to reflect the Canadian donor population of 418,700 individuals.

The expected number of donors eligible for ferritin tests under Scenario 2 is presented in Table 2. In the baseline case (Scenario 1), 122,809 ferritin tests would have been administered, under current IDI intervals of 56 days for males and 84 days for females if testing were in place. Simulation results show a slight reduction from baseline by 4,419 tests (*95% CI Half Width: 422*) in the aggregate number of donors requiring a ferritin test, if an extended IDI is adopted. This represents a reduction in ferritin tests of 3.6% (*95% CI Half Width: 0.3%*) from Scenario 1.

Table 3 shows simulation results for the annual number of donors expected to receive a ferritin test value below 26 µg/L. The simulation

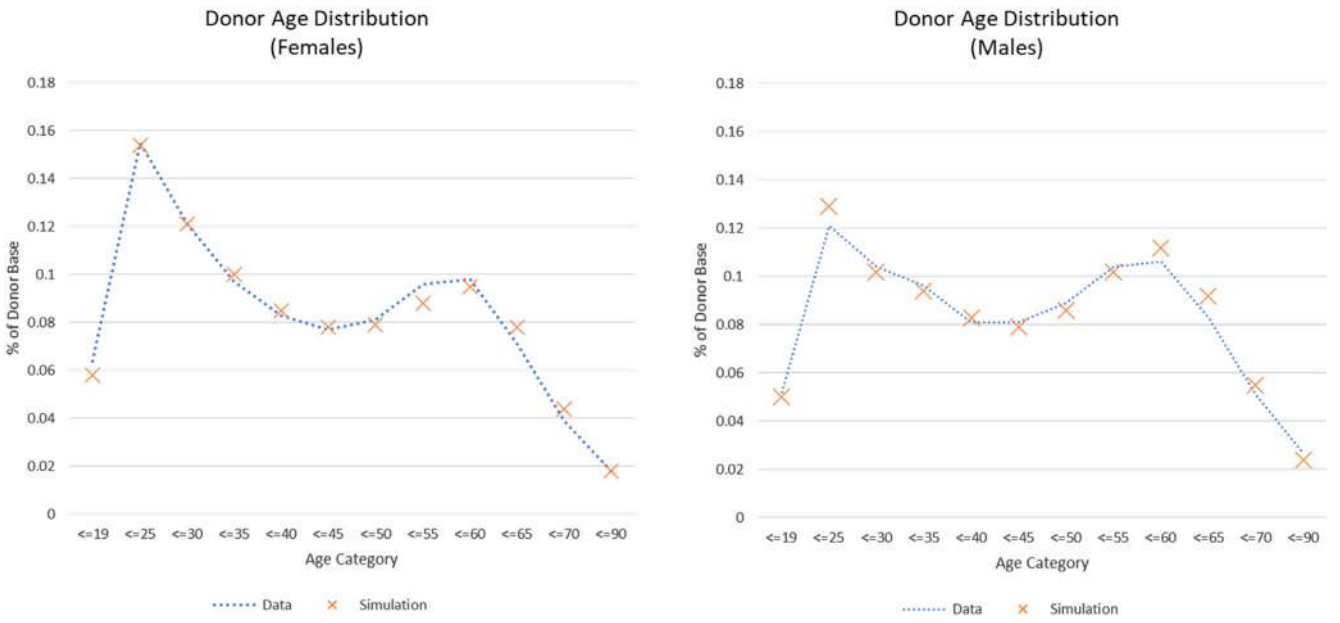


FIGURE 5 Comparison of age distribution for donors (female and male) against historical data for verification purposes. In both cases, a χ^2 test indicated that there was no reason to believe that the simulated results differed from that of the historical data [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Simulation results showing annual RBC donations, broken down by gender

	Donations					
	Scenario 1	**Scenario 2**	**Mean change**	**CI half width change**	**% change**	**CI half width % change**
Females	335,876	331,100	4776	1402	1.4%	0.4%
Males	453,591	433,904	19,687	438	4.3%	0.1%
Total	789,467	765,004	24,463	1472	3.1%	0.2%

Note: Mean differences, standard deviation, and 95% confidence interval half widths are given. A confidence interval may be constructed as the mean +/− the confidence interval half width.

TABLE 2 Simulation results showing annual number of donors eligible for ferritin testing

	Ferritin tests					
	Scenario 1[a]	**Scenario 2**	**Mean change**	**CI half width change**	**% change**	**CI half width % change**
Females	49,073	48,299	774	245	1.6%	0.5%
Males	73,736	70,091	3645	361	4.9%	0.5%
Total	122,809	118,390	4419	422	3.6%	0.3%

[a]The model is simulating the number of tests that would meet the criteria for testing if we had testing in place at the time with no deferral for low ferritin.
Note: Mean differences, standard deviation, and 95% confidence interval half widths are given. A confidence interval may be constructed as the mean +/− the confidence interval half width.

results suggest 55,691 low ferritin values would be detected currently if donors were tested every fifth donation and existing IDIs were in place. A total of 52,726 low donor values would be expected, if donors were tested every fifth donation and a 6-month IDI was adopted for donors with low or very low ferritin, representing a decrease of 2965 (*95% CI Half Width: 261*) deferrals or 5.3% (*95% CI Half Width: 0.5%*) from the baseline.

Scenarios 3A-3C (low ferritin result influences donor behaviour)

Simulation results show that both post-test donor retention and post-test donation frequency both have a significant effect on whole blood donations, the availability of blood products and the number of new donors that need to be recruited to maintain the donor pool. Scenario

TABLE 3 Simulation results showing annual number of donors deferred due to a low ferritin level

	Deferred donors					
	Scenario 1[a]	Scenario 2	Mean change	CI half width change	% change	CI half width % change
Females	30,657	30,020	637	249	2.1%	0.8%
Males	25,034	22,706	2328	869	9.3%	3.5%
Total	55,691	52,726	2965	261	5.3%	0.5%

[a]The model is simulating the number of donors that would meet the criteria for deferral if we had testing in place at the time.
Note: Mean differences, standard deviation, and 95% confidence interval half widths are given. A confidence interval may be constructed as the mean +/− the confidence interval half width.

TABLE 4 Annualized whole blood donations by scenario

	Scenario 1 Current IDI	Scenario 2 Extended IDI	Scenario 3A Extended IDI and reduced donor retention	Scenario 3B Extended IDI and reduced donation frequency	Scenario 3C Extended IDI, reduced donor retention and donation frequency
Females	335,876	331,100	278,119	285,483	260,004
Males	453,492	433,904	436,404	352,300	394,872
Total	789,368	765,004	714,523	637,783	654,876
Change	—	24,364	74,845	151,585	134,492
CI half width change		1471	2651	3223	4080
% Change		3.1%	9.5%	19.2%	17.0%
CI half width % change		0.2%	0.1%	0.2%	0.2%

TABLE 5 Annualize numbers of new donors recruited by scenario

	Scenario 1 Current IDI	Scenario 2 Extended IDI	Scenario 3A Extended IDI and reduced donor retention	Scenario 3B Extended IDI and reduced donation frequency	Scenario 3C Extended IDI, reduced donor retention and donation frequency
Females	63,015	62,540	85,416	62,161	80,946
Males	53,642	53,303	72,898	52,790	68,555
Total	116,656	115,843	158,315	114,951	149,501
Change	—	−813	41,658	−1706	32,845
CI half width change		833	990	1203	1523
% Change		−0.7%	36.0%	−1.1%	28.6%
CI half width % change		−1.9%	2.3%	2.8%	3.5%

Note: Comparisons are reported relative to scenario 1.

results are shown in Tables 4 and 5 and are made in comparison to Scenario 1.

Scenario 3A

If only donor retention changes following a low ferritin test result, a decrease in collections of 74,845 (*95% CI Half Width: 2651*) units or 9.5% (*95% CI Half Width: 0.1%*). Broken down by gender, this is a 17.2% (*95% CI Half Width: 0.2%*) decrease for females and a 3.7% (*95% CI Half Width: 2.3%*) decrease for males. In this scenario, it is expected that a total of 158,315 new donors would need to be recruited to maintain the donor pool size. This is an increase of 41,658 new donors (*95% CI Half Width: 990*), or 36.0% (*95% CI Half Width: 2.3%*), over the current case (Scenario 1).

Scenario 3B

If only donation frequency changes following a low ferritin test, a decrease in donations of 151,585 (95% CI Half Width: 3223) units or 19.2% (*95% CI Half Width: 0.2%*) is suggested; a 15.0% (*95% CI Half Width: 0.26%*) decrease for females and a 22.3% (*95% CI Half Width: 2.4%*) for males can be anticipated. A total of 114,951 new donors

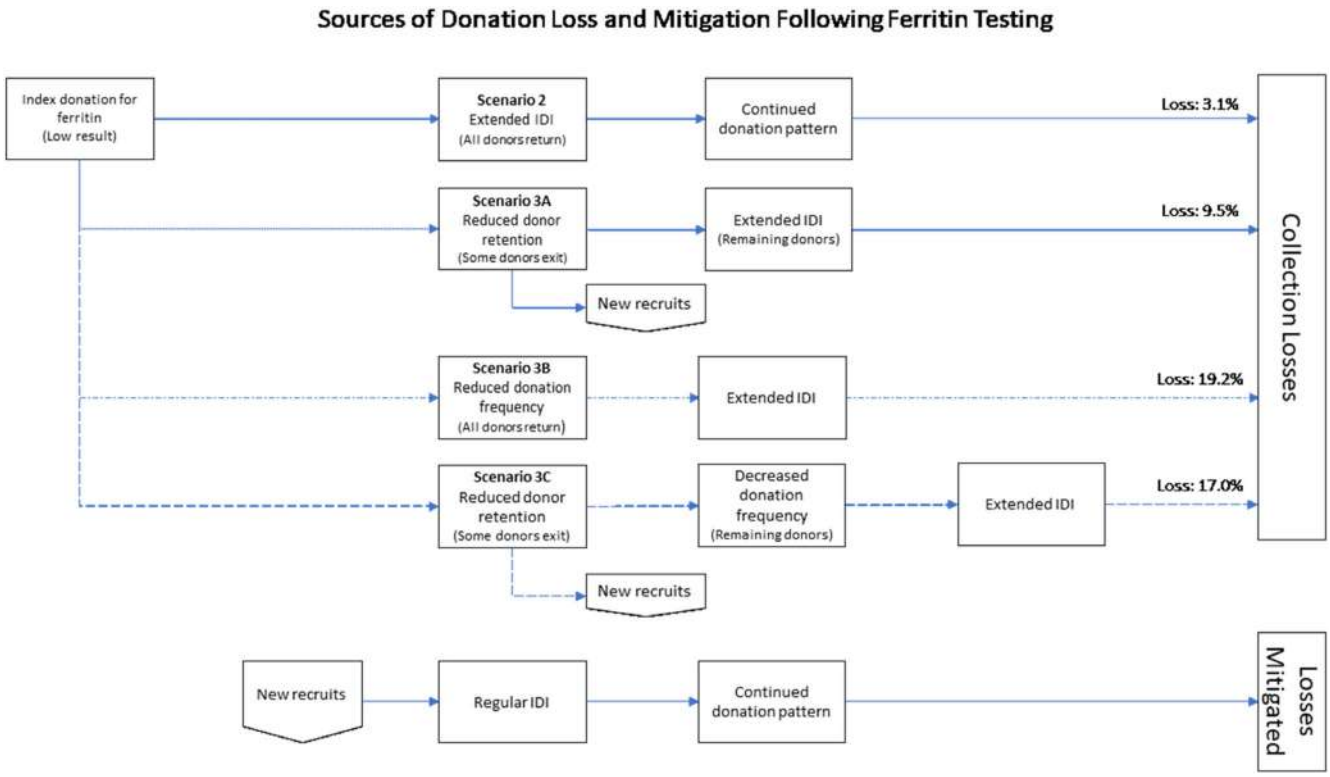


FIGURE 6 Schematic representation of sources donation losses and possible mitigation following a low ferritin test result [Colour figure can be viewed at wileyonlinelibrary.com]

were recruited under this scenario. This is a decrease of 1706 new donors (*95% CI Half Width: 1203*), or 1.1% (*95% CI Half Width 2.8%*) in comparison to Scenario 1.

Scenario 3C

When both donor retention and donation frequency change after a low ferritin test, a decrease in collections of 134,492 (*95% CI Half Width: 4080*) units or 17.0% (*95% CI Half Width: 0.2%*) is suggested. This corresponds to a 22.6% (*95% CI Half Width: 0.2%*) decrease for females and a 12.9% (*95% CI Half Width: 0.1%*) decrease for males. A total of 149,501 new donors were recruited under this scenario. This is an increase of 32,845 new donors (*95% CI Half Width: 1523*), or 28.6% (*95% CI Half Width: 3.5%*) over the current case.

Scenarios 3A–3C results show that changes to donor retention and donation frequency following a low ferritin test impact both annual donations and the number of new donors needed to maintain the donor pool. Moreover, there is a clear interaction between donor retention and donation frequency, particularly for males, following a low ferritin test. The impact of a low ferritin test on repeat female donors is notable under all scenarios, but less substantial. See Figure 6 for a schematic representation of donation losses, donation loss mitigation, and necessary changes to donor recruitment following a low ferritin test result.

DISCUSSION

Supplementing haemoglobin-based donor qualification with ferritin testing will decrease the number of blood units that may be drawn from a population.

Table 4 shows that whole blood donations will decrease by 3.1% (*95% CI Half Width:0.2%*) if IDIs are extended to 6 months, for individuals with low ferritin levels, from existing IDIs.

Of greater impact on the availability of blood products, however, is the impact of ferritin test results on individual behaviour. Operational data from Canadian Blood Services, when merged with the study data from Goldman et al. [4, 10], indicates that donor retention decreases when a low ferritin test result is made known to a donor and that, if donors elect to return, their donation frequency decreases, and/or the time their make their first post-test donation increases. When donor behaviour is incorporated into model results, declines in blood collections were more significant than losses due strictly to increased inter-donation interval (see Table 4); a decrease of 17.0% (*95% CI Half Width: 0.2%*) was observed when both donor retention and donation frequency change following an individual's low ferritin test (Scenario 3C). If repeat donors receiving a low ferritin test result remain and decrease their frequency of donation (Scenario 3B), the average donation frequency in the Canadian donor pool declines from 1.64 (*95% CI Half Width: 0.01*) donations per person per year to 1.32 (*95% CI Half Width: 0.01*) donations per person per year, resulting in an overall decline in collections of 19.2% (*95% CI Half Width: 0.2%*).

Accordingly, it may be determined that factors that decrease donation frequency amongst repeat donors have the greatest impact on blood product availability.

However, the simulation also shows that ferritin testing impacts the number of new donors that must be recruited to maintain the donor pool; factors that influence donor retention, rather than donation frequency, have the greatest impact on the number of new donors recruited in the simulation. When changes to donor behaviour are included in the model, significant modifications in the donor recruiting patterns were observed. If donor retention decreases (Scenario 3A), following the result of a low ferritin test, 41,659 new donors (36.0% increase over baseline) are required. If donor retention does not change, but donor frequency deceases (Scenario 3B), new donor recruiting actually decreases by 1705 (1.5% decrease from the baseline). In the case that both donor retention and donation frequency decrease following the implementation of ferritin testing (Scenario 3C), new donor recruiting increases by 32,845 (28.6% increase over baseline). Thus, while changes to donor retention were seen to have less impact on product availability in the simulation than changes in donor frequency, decreased donor retention results in greater recruiting pressures.

Note that in this study, a fixed population of 10,000 donors is maintained and thus all departing donors are replaced in our simulation. As a result, losses due to donors who remain in the donor pool, but reduce their donation frequency, can be seen to significantly impact blood product availability. Nevertheless, increased donor enrolment will be needed to make up for these lost collections. Moreover, factors that affect blood donor retention, while having less impact on product availability, also trigger increases in the need for donor recruiting. Thus, the simulation shows that blood agencies will be required to recruit substantial numbers of new donors and/or encourage increased donation frequency amongst repeat donors to maintain blood product availability following the implementation of ferritin testing for donors. In short, any change to donor behaviour that decreases donation frequency or donor retention, will require additional new donor enrolment.

The impact of changes in the donation behaviour reported here are extrapolated from changes in donor behaviour from a pilot study conducted by Goldman et al. [4] and thus results in an operational environment may vary. It is possible that return rates might be better than assumed in this study if further retention and education efforts were deployed. Further, the data available does not extend far enough in time to discern if changes to donor behaviour following a low ferritin test are permanent; we assume so in our model, but it is possible that donors may revert to their previous donation pattern. Finally, our model specifically excludes haemoglobin deferrals. It is known [7] that haemoglobin deferrals decrease amongst donors returning from an extended IDI and therefore, there may be some mitigation of losses created by donors delaying their return following a low ferritin result. Nevertheless, given the degree of change seen in our results is evident that changes to donor behaviour will have a much greater effect on blood product collections than mandated changes to inter-donation intervals.

A safe, adequate supply of blood is needed to ensure the health of recipients in need. It is, however, imperative that donors' health be guarded. Ferritin testing for donors provides greater sensitivity, when compared to haemoglobin testing. However, this testing will reduce the amount of blood that can be collected from a given donor population. Reductions will occur due to a mandated extension in inter-donation intervals and changes in donor behaviour. Simulation results suggest a reduction of 3.1% (*95% CI Half Width:0.2%*) in collections due to extended inter-donation intervals and reductions of at least 9.5% (*95% CI Half Width: 0.1%*), and possibly as much as 19.2% (*95% CI Half Width: 0.2%*) if donors with low ferritin levels either exit the donor pool or remain and reduce their donation frequency. In addition to recruiting needed to make up for decreased donation frequency amongst return donors, declines in donor retention triggered could also increase the need for new donors by as much as 36.0% (*95% CI Half Width: 2.3%*).

We conclude, therefore, that programs to manage donor behaviour must be deployed along with ferritin testing. The results from this study show that while extensions to mandated IDI will affect whole blood collections, the impact created by continuing changes in donor behaviour following a low or very low ferritin test are much more significant for the blood supply chain. Thus, blood agencies must proactively manage their donor base to ensure that individuals' iron stores are not depleted through blood donation activities and that, if low iron stores are detected, donors are motivated to modify their lifestyle and, when healthy, return to the donor pool. A plan for recruitment of new donors and encouraging more donations from repeat donors must accompany the implementation of ferritin testing to offset decreased donation frequency and/or decreased donor retention.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada Discovery Grant program and by Canadian Blood Services.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest with regards to this work.

ORCID

John T. Blake https://orcid.org/0000-0003-0617-8996
Sheila F. O'Brien https://orcid.org/0000-0002-5332-2789
Mindy Goldman https://orcid.org/0000-0001-9904-9952

REFERENCES

1. Finch C, Cook J, Labbe R, Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. Blood. 1977;50:441–7.
2. Newman B. Iron depletion by whole-blood donation harms menstruating females: the current whole-blood collection paradigm needs to be changed. Transfusion. 2006;46:167–1681.
3. Newman B. Improving the US blood supply and blood donation safety for both women and men. Transfusion. 2008;48:1032–5.
4. Goldman M, Uzicanin S, Scalia V, O'Brien S. Iron deficiency in Canadian blood donors. Transfusion. 2014;54:775–9.

5. Cable R, Glynn S, Kiss J, Mast A, Steele W, Murphy EWD, et al. Iron deficiency in blood donors: the REDS-II donor iron status evaluation (RISE) study. Transfusion. 2012;52:702–11.
6. Milman N, Kirchoff M. Influence of blood donation on iron stores assessed by serum ferritin and haemoglobin in a population suver of 1433 Danish males. Eur J Haematol. 1991;47:134–9.
7. Vinkenoog M, van den Hurk K, van Kraaij M, van Leeuwen M, Janssen M. First results of a ferritin-based blood donor deferral policy in The Netherlands. Transfusion. 2020;60:1785–92.
8. O'Meara A, Infanti L, Stebler C. The value of routine. Transfusion. 2012;52:1566–75.
9. Gorlin J, Katz L, Elsmore D, Kirbach K, Erickson Y, Hove A, et al. Prevelance of blood donor iron deficiency and feasibility of ferritin-based iron replacement: a blood collection agency-based study. Vox Sang. 2016;111:206–8.
10. Goldman M, Uzicanin S, Osmond L, Yi Q, Scalia V, O'Brien SF. Two year follow-up of donors in a large national study of ferritin testing. Transfusion. 2018;58:2868–73.
11. Spekman M, Ramondt S, Sweegers M. Whole blood donor behavior and availability after deferral: consequences of a new ferritin monitoring policy. Transfusion. 2020;61:1112–21.
12. Goldman M, Yi Q, Steed T, O'Brien S. Changes in minimum hemoglobin and interdonation interval: impact on donor hemoglobin and donation frequency. Transfusion. 2019;59:1734–41.
13. Birgegard G, Hogman C, Killander A, Wide L. Serum ferritin levels in males blood donors. Vox Sang. 1978;34:65–70.
14. Blake J. Modelling the Need for New Blood Donors Following a Change in Deferral Period. IJCIEOM 2018: Industrial Engineering and Operations Management, Lisboa, 2018.
15. Spencer B, Johnson B, Wright D, Kleinman S, Glynn S, Cable R, et al. Potential impact on blood availability and donor iron status of changes to donor hemoglobim cutoff and interdonation intervals. Transfusion. 2016;56:1994–2004.
16. Walpole RE, Myers RH, Myers SL, Ye KE. Probability and Statistics for Engineers and Scientists. 8th Edition, Upper Saddle River, NJ: Pearson press; 2007.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Blake JT, O'Brien SF, Goldman M. The impact of donor ferritin testing on blood availability in Canada. Vox Sang. 2021;1–10.

Received: 21 January 2021 | Revised: 7 April 2021 | Accepted: 7 April 2021

DOI: 10.1111/vox.13118

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Biochemical and cellular markers differentiate recovered, in-line filtered plasma, and plasma obtained by apheresis methods

Jürgen Siekmann[1] | Alfred Weber[1] | Christoph Bauer[2] | Peter L. Turecek[1]

[1]Plasma-Derived Therapies R&D, Baxalta Innovations GmbH, part of Takeda, Vienna, Austria

[2]Global Manufacturing Science Plasma, Baxter AG, part of Takeda, Vienna, Austria

Correspondence
Peter L. Turecek, Plasma-Derived Therapies R&D, Baxalta Innovations GmbH, part of Takeda, Vienna, Austria.
Email: peter.turecek@takeda.com

Funding information
Baxalta Innovations GmbH

Abstract

Background and Objectives: Assessment of plasma quality often focuses on the common safety tests for minimizing the risk of transmitting blood-borne pathogens. Little attention is paid to the possible quality attributes that ensure a consistent biochemical composition of plasma for fractionation. We therefore investigated the suitability of selected biochemical and haematological attributes that could be used as markers of plasma quality obtained by different separation and pre-treatment procedures.

Material and Methods: We characterized six plasma types, including source plasma, plasma recovered by classic means and in-line filtered plasma, by determining the analytical attributes protein content, coagulation factors and markers of coagulation, contact and complement activation. Residual cell content and cell-specific variables were also measured.

Results: We found relevant differences between the plasma types in complement activation, as indicated by C3a measurements, while thrombin antithrombin complex values and, to a minor extent, activated factor XII concentrations indicated only moderate differences in activation levels of coagulation and contact systems. The most striking differences, however, were detected in residual cell content and concentrations of the platelet-associated proteins, platelet factor 4 and β-thromboglobulin. We showed that leucocyte reduction filters disrupt cells. This includes platelets, thereby releasing the platelet-associated proteins platelet factor 4 and β-thromboglobulin, and leucocytes as demonstrated by the release of elastase from polymorphonuclear leucocytes. Furthermore, the filtration processing of whole blood can lead to activation of the complement system.

Conclusion: Our results show that biochemical and cellular surrogate markers are valuable discriminators of plasma types.

KEYWORDS
cell disruption, plasma for fractionation, plasma quality, platelet-associated proteins, platelet factor 4, PMN elastase, β-thromboglobulin

INTRODUCTION

Human plasma is a valuable raw material that produces functional proteins used in the therapy of coagulation, haematological and immunological disorders [1–4]. The quality of this starting material and the final products derived from it depends on two main factors: the blood or plasma of donors [5], and plasma preparation method [3, 6–9]. Current quality control of plasma for fractionation focuses on minimizing the risk of transmitting infectious diseases caused by blood-borne viruses and bacteria [6, 10–12], with testing done on each single plasma unit. Apart from the pharmacopeial requirements regarding, amongst others, residual blood cells, total protein content and minimum content in coagulation factors, where testing is not done on each single unit, little attention has been paid to the biochemical variables that ensure a consistent biochemical plasma composition. Types of plasma vary according to the preparation technique used, with several different types available from plasmapheresis units and transfusion services.

Source plasma [13] is defined as the fluid portion of human blood collected by plasmapheresis intended for further manufacturing use and can be prepared by automated plasmapheresis. This procedure can be carried out, for instance, by the Autopheresis-C or Aurora system (Fresenius Kabi) [14–16], which uses spinning membrane technology, or the PCS-2 system (Haemonetics, Massachusetts) [14, 17], which uses centrifugation.

Plasma can also be recovered by centrifugation from whole blood donations, with and without leucoreduction. Leucoreduction is achieved by filtration. Plasma recovered with leucoreduction is also termed in-line filtered plasma. All the different filter systems used in leucocyte reduction steps aim to reduce the leucocyte load in erythrocyte concentrates and minimize leucocyte-associated side effects [18–21], and the possibility of blood components transmitting prions that cause a variant form of Creutzfeldt-Jakob disease (vCJD) [22, 23].

The various plasma types produced by these methods are the raw and starting material for plasma fractionators, which commonly process plasma into several different products. However, different plasma production methods could have an impact on the composition of the plasma obtained. Therefore, to achieve a controlled, sustained quality of these products, it would clearly be beneficial if the quality attributes for the starting material could be defined. Up until now, apart initiating the common serological and nucleic acid amplification tests [24, 25], there have been few attempts to define the attributes that define plasma quality. Thus, Burnouf et al. [26] compared protein composition and activation markers in plasma collected by three apheresis procedures, while Runkel et al. investigated the quality of apheresis and recovered plasma [27], and the impact of whole blood in-line filtration [28]. The biochemical analyses were conducted on single plasma donations, and were similar in all three studies, focusing mainly on the measurement of various activation markers.

To advance knowledge on this topic, we investigated whether different plasma preparation techniques influence the function and morphology of the plasma obtained. In particular, we compared selected biochemical and haematological attributes using single plasma donations and plasma production pools from different sources. In addition, data on the content of residual cells and cell debris were generated as described [29]. A similar protocol was used to compare source plasma quality in three apheresis protocols [30].

MATERIALS AND METHODS

Plasma samples and freezing protocol

We used six types of plasma obtained by different preparation methods: two were source plasma obtained by Autopheresis-C (Fresenius Kabi) and by the PCS-2 system (Haemonetics), respectively, one was recovered by the classic method (hereafter termed recovered plasma) and three were in-line filtered from whole blood that had been subjected to different filter systems to reduce the leucocyte load. These three different in-line filtered plasma types were designated type A, B, and C. Our aim was to have at least 10 individual samples from each plasma type. Accordingly, 10 individual units each of plasma types Autopheresis-C, Haemonetics, A and B were analysed, while 12 and 26 individual units were available from recovered and type C plasma, respectively. The plasma samples were obtained from Austrian BioLife plasmapheresis centres and from other European Baxter AG plasma suppliers. Source plasma (Autopheresis-C and Haemonetics PCS-2) was collected with 6% trisodium citrate (v/v) as an anticoagulant by use of 4% (w/v) trisodium citrate solution. A citrate-phosphate-dextrose solution was used as an anticoagulant for recovered and in-line filtered plasmas.

All samples were aliquoted directly after preparation (1-ml samples), frozen at −70°C overnight and subsequently stored at −20°C. Before testing, the samples were thawed at +37°C. Some selected samples were investigated immediately after preparation. These freshly prepared samples were transported to the laboratory at +4°C and testing started within 2 h of their preparation.

Plasma pool samples (source plasma, $n = 36$, recovered plasma, $n = 24$ and in-line filtered plasma, $n = 22$) consisting of 2000 to 6000 single plasma donations were obtained from the standard manufacturing process at Baxter AG, Vienna, Austria (part of Takeda).

Assays for assessment of biochemical attributes

The protein concentration was measured by Biuret assay [31]. Factor VIII (FVIII) activity was determined by chromogenic assay [32] using reagents supplied by Technoclone (Vienna, Austria). Von Willebrand factor ristocetin cofactor (VWF:RCo) was determined by measuring the agglutination of formaldehyde-fixed human platelets using the standard procedure [33], with a 570-VS whole blood aggregometer (Chrono-Log, Havertown, Pennsylvania) equipped with a chart recorder. The antigen concentration of VWF (VWF:Ag) was determined with sandwich ELISA Asserachrom® VWF (Boehringer Mannheim, Germany), while VWF collagen-binding (VWF:CB) activities were determined by Immunozym VWF:CBA [34] (Technoclone, Vienna, Austria). VWF multimers were analysed by sodium dodecyl sulfate (SDS)-agarose gel electrophoresis

using a 1% gel according to Ruggeri and Zimmerman [35], with minor modifications [36]. Thrombin-antithrombin complex (TAT) and prothrombin fragments F1 + 2 concentrations were determined by commercially available sandwich ELISAs (Siemens, Vienna, Austria), activated coagulation factor XII (FXIIa), a marker for the activation of the contact activation system, was determined by a commercially available sandwich ELISA system (Shield Diagnostics, Dundee, UK). Complement activation was determined by measuring C3a concentrations with a commercially available sandwich ELISA system (Quidel, Mountain View, California). Lactate dehydrogenase (LDH) was measured by a photometric standard method at 25°C (Boehringer Mannheim GmbH, Mannheim, Germany).

Determination of residual cell content

Residual cells (platelets, leucocytes, erythrocytes, and erythrocytes ghosts) were quantified in frozen samples by flow cytometric (FACS) analysis using a no-lyse/no-wash preparation procedure [37]. The manufacturer's instructions were followed for leucocyte enumeration with LeucoCount (BD Biosciences, Sunnyvale, California). To quantify platelets and erythrocytes, 100 µl of plasma were added to TrueCount tubes (BD Biosciences) and incubated with 10 µl of a monoclonal antibody cocktail against CD42a (FITC, BD Biosciences) and glycophorin-A (PE, Dako, Glostrup, Denmark). After 30 min of incubation at 4°C, 300 µl PBS were added before measurement on a FACSCalibur (BD Biosciences). Platelets and erythrocytes were subsequently measured separately from the same tube. Cell debris signals were removed. Logarithmic amplification was used for all variables. Data were evaluated by multi-parameter analysis using the Paint-A-Gate software (BD Biosciences).

Blood cell-associated proteins

Platelet factor 4 (PF4) and β-thromboglobulin (bTG) concentrations were determined in samples frozen and thawed one time by commercially available sandwich ELISAs obtained from STAGO Diagnostics (Roche Diagnostics, Mannheim, Germany). In a separate study, bTG and PF4 were released from platelets. Briefly, platelets were prepared from whole blood by a standard in-house procedure and suspended in physiological HEPES-NaCl buffer, pH 7.35. The platelet content was measured with the Sysmex K-1000 cell counter (Toa Medical Electronics, Kobe, Japan). Suspensions were adjusted to contain 125,000, 250,000 and 500,000 platelets/µl and stabilized by the addition of 5% (w/v) human serum albumin (Baxter AG, Vienna, Austria). The suspensions were deep frozen at −70°C and lyophilised in aliquots of 1 ml. After lyophilisation, the samples were reconstituted with the appropriate amount of water and the bTG and PF4 concentrations determined. Elastase from polymorphonuclear leucocytes was determined in complex with alpha1-proteinase inhibitor by use of an ELISA (Merck, Darmstadt, Germany).

Platelet function analysis with platelet function analyser

The platelet function analyser PFA-100/200 (Siemens, Vienna, Austria) is a high shear stress in vitro system for the detection of platelet dysfunction [38, 39]. The instrument simulates the process of primary haemostasis by aspirating the whole blood sample from a reservoir through a capillary and a microscopic aperture cut in the membrane. This membrane is coated with type I collagen and either epinephrine (EPI) or adenosine 5′-diphosphate (ADP) as a stimulating agent. The time needed to obtain full occlusion of the aperture is defined as closure time. Aliquots of 800 µl of each, taken from the three samples of citrated whole blood obtained from two independent blood donations before and after in-line filtration with in-line filters type A, were applied to collagen-EPI and collagen-ADP cartridges. Closure times were determined by the standard procedure according to the manufacturer's instructions and were expressed as means of six values (three aliquots of two blood donations). The samples' platelet content was determined as described above.

Statistical evaluation

Statistical data evaluation was done with one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism version 8.0.0 for Windows. The significance level was 0.05.

RESULTS

Biochemical variables in different plasma types

Our first step was to analyse biochemical attributes in analytical samples of different plasma types that had been subjected to freezing and thawing. These data are summarized in Table 1. ANOVA revealed significant differences between total protein levels: The lowest protein values were determined for the two source plasma types Autopheresis-C and Haemonetics PCS-2. This was to be expected for source plasma collected from donors undergoing intensified plasmapheresis [40, 41]. In addition, comparison of recovered and the in-line filtered plasmas showed that the level of total protein is not affected by the different in-line filtration procedures (Supplemental Material, Figure 1).

The FVIII activity levels of the different plasma types are summarized in Table 1 and Figure 1a, which show the significant differences found between the individual plasma types. Actual values ranged from 0.79 IU/ml (In-line Filtered Plasma, Type B) to 1.19 IU/ml (recovered plasma), but with high variability between the individual units. [Correction added on 06 September 2021 after first online publication: The range of actual values of individual plasma types was corrected to "0.83 IU/ml (Autopheresis-C)" in the preceding sentence.]. Their levels did not differ significantly between the plasma types, with VWF:RCo showing slightly higher differences (Table 1). All data

TABLE 1 Biochemical variables in different plasma types

Attribute	Source Plasma		Recovered	In-line Filtered Plasma			ANOVA Results[a] ($\alpha = 0.05$)	Reference Range
	Auto-C ($n = 10$)	Haemonetics ($n = 10$)	Classic ($n = 12$)	Type A ($n = 10$)	Type B ($n = 10$)	Type C ($n = 26$)		
Protein (mg/ml)	51.1 ± 2.1	55.9 ± 2.4	58.0 ± 4.1	58.5 ± 2.9	63.6 ± 4.8	56.4 ± 4.3	$p < 0.0001$	60–75
FVIII:C (IU/ml)	0.83 ± 0.29	1.00 ± 0.29	1.19 ± 0.38	0.90 ± 0.15	0.79 ± 0.18	0.99 ± 0.27	$p = 0.0392$	0.6–1.4
VWF:Ag (IU/ml)	1.21 ± 0.46	1.32 ± 0.36	1.02 ± 0.27	1.28 ± 0.22	1.22 ± 0.40	1.04 ± 0.49	$p = 0.2685$	0.6–1.4
VWF:CB (U/ml)	0.96 ± 0.41	0.98 ± 0.23	0.94 ± 0.25	1.05 ± 0.21	0.99 ± 0.28	0.92 ± 0.44	$p = 0.9408$	0.6–1.4
VWF:RCo (IU/ml)	0.82 ± 0.34	0.94 ± 0.23	0.94 ± 0.32	1.31 ± 0.31	0.99 ± 0.26	0.98 ± 0.41	$p = 0.0501$	0.6–1.4
TAT (ng/ml)	1.4 ± 0.7	4.7 ± 11.2	17.4 ± 33.8	3.3 ± 2.9	1.6 ± 1.5	2.4 ± 1.4	$p = 0.0471$	1.0–4.1
F1 + 2 (nmol/l)	0.9 ± 0.4	0.9 ± 0.5	1.0 ± 0.4	1.2 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	$p = 0.0337$	0.4–1.1
FXIIa (ng/ml)	1.9 ± 0.3	1.6 ± 0.8	1.8 ± 0.9	2.0 ± 0.4	1.6 ± 0.3	2.5 ± 1.2	$p = 0.0187$	< 1–2.9
C3a (ng/ml)	4914 ± 1059	250 ± 78	299 ± 133	245 ± 94	621 ± 343	2307 ± 1489	$p < 0.0001$	26–146[b]

Note: Remarks: Means ± standard deviations are shown.

[a]One-way ANOVA was performed followed by Tukey's multiple comparisons test using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California. Results obtained for the individual comparison of groups with the Tukey's multiple comparison test are shown in Supplemental Material.

[b]The reference range for the C3a concentration was determined in EDTA plasma.

showed that the in-line filtration procedures did not affect the VWF levels. Moreover, detailed analysis of the different VWF variables indicated that the multimeric structure of VWF was not affected in the different samples with one exemption: Plasma obtained by the Haemonetics PCS-2 system was devoid of the high-molecular weight multimers, as demonstrated in Figure 1b (lane 2). However, this was not reflected by the functional VWF assays VWF:CB and VWF: RCo, probably as a consequence of their variability or their lower sensitivity in detecting high-molecular weight VWF multimers compared with multimer analysis. Despite this small variation in the different individual plasma samples, there was no evidence that the in-line filtration process affected the multimer composition of VWF.

The concentrations of the coagulation activation marker prothrombin fragment F1 + 2 and TAT differed significantly between the plasma types (Table 1, Figure 2). The lowest average TAT value was found for the source plasma obtained by the Autopheresis-C system, and highest for the recovered plasma (Figure 2a). The large differences could, however, be partly due to outlying abnormal values of single donors that can originate from the venepuncture applied and which is known to lead to activation of the coagulation system. We noticed that TAT concentrations ranged widely (from 0.7 to 122 ng/ml) in the recovered plasma. The values for FXIIa, a marker of contact activation, also differed significantly, mainly between Haemonetics and plasma type C (Supplemental Material Figure 8). FXIIa concentrations higher than 4 ng/ml were measured which were probably due to in-line filtration with the filter for type C (Figure 2c). Significant differences were found for the C3a concentrations. The highest mean concentrations of C3a, indicative of complement activation, were obtained for source plasma prepared with the Autopheresis-C system (4914 ng/ml, Figure 2d). Plasma obtained by the Haemonetics system and by the in-line filtered plasma type A had essentially the same concentration (range 200–300 ng/ml). Slightly higher values were measured for in-line filtered plasma type B (approximately 600 ng/ml) and considerably higher values for in-line filtered plasma type C (2307 ng/ml). We conclude that filtration processes during plasma preparation can induce complement activation, dependent on the filter type used.

As mentioned above, we were especially interested in determining the residual cell contents of different plasma types. For these measurements, we used a FACS procedure [37] which allows linear detection of as low as 6 platelets/μl, 8 erythrocytes/μl, and 1 leucocyte/μl. We found large differences between the plasma types in the platelet content of samples that had been frozen and thawed again. Clearly, this procedure could impact the results obtained. Assuming that these possible effects would affect all samples to the same extent, a comparative data evaluation seemed to be justified. The highest residual cell contents (mean approximately 15,000 cells/μl; Figure 3) were found for plasma obtained with the Haemonetics system and recovered plasma, both of which were prepared by a process based on centrifugation. By contrast, the three plasma types prepared from in-line filtered whole blood, which were also separated by centrifugation, had considerably lower platelet content. We therefore hypothesise that the platelets are affected by the in-line filtration procedure. Plasma prepared with Autopheresis-C had a very low platelet content, which was close to the detection limit of the FACS procedure.

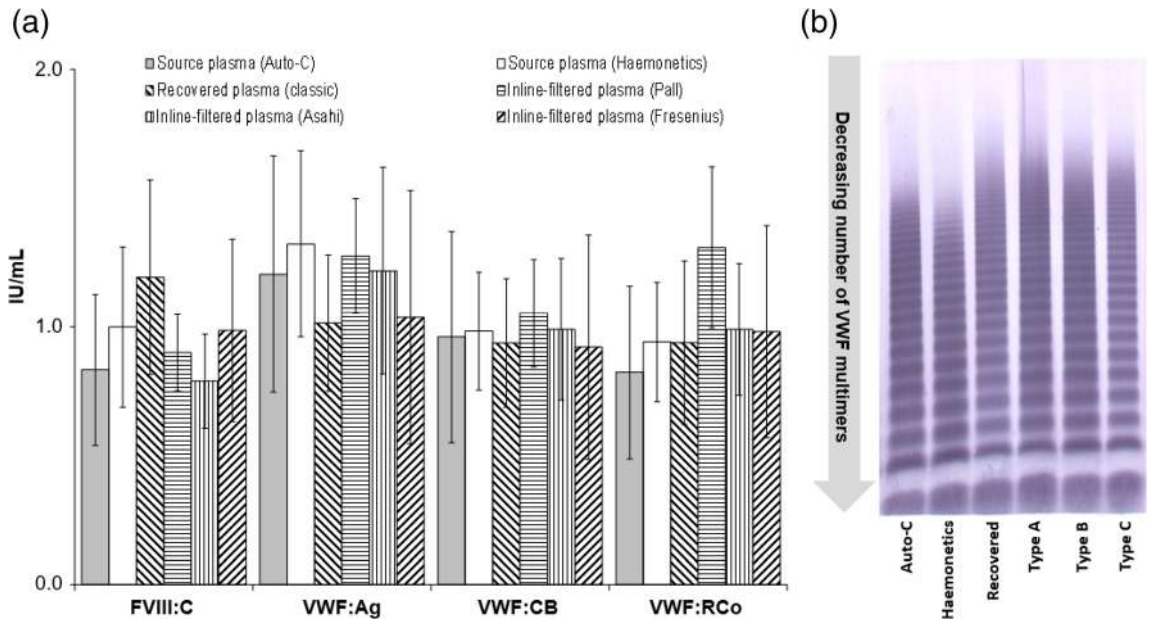


FIGURE 1 FVIII and VWF-related attributes in different plasma types. (a) The mean FVIII activities and the mean VWF-related attributes VWF:Ag, VWF:Co and VWF:CB concentrations for the six different plasma types. Error bars mark the single SD. The following numbers of single plasma samples were averaged: Source plasma (Autopheresis C), $n = 10$; source plasma (Haemonetics), $n = 10$; recovered plasma (classic), $n = 12$; in-line filtered plasma type A, $n = 10$; in-line filtered plasma type B, $n = 10$; in-line filtered plasma type C, $n = 26$. (b) The analysis of the multimeric composition of VWF with a 1% agarose gel

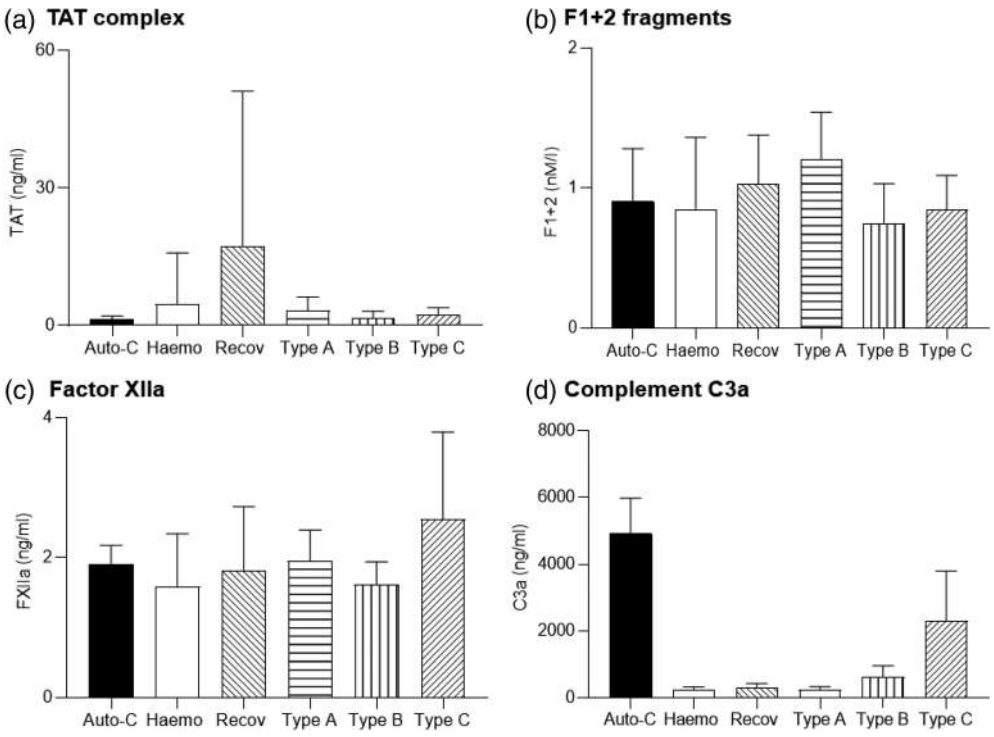


FIGURE 2 Activation markers in different plasma types. The mean concentrations of the activation markers TAT (a), prothrombin F1 + 2 (b), FXIIa (c) and complement C3a (d) are shown. Error bars mark the single SD. The following numbers of single plasma samples were averaged: Source plasma (Autopheresis C), $n = 10$; source plasma (Haemonetics), $n = 10$; recovered plasma (classic), $n = 12$; in-line filtered plasma type A, $n = 10$; in-line filtered plasma type B, $n = 10$; in-line filtered plasma type C, $n = 26$

We also found that recovered plasma had the highest leucocyte content (approximately 50 cells/μl, Figure 3). All the other plasma types showed a very low leucocyte content (<10 cells/μl). No intact erythrocytes could be detected in the different plasma preparations, although we did find damaged cells, referred to as "ghosts", to a varying extent (Figure 3) [37]. The two source plasma types had the lowest content. Recovered plasma and the three in-line filtered plasmas had substantially higher values. Measurement of the residual cell content demonstrated that plasma prepared by the Autopheresis-C system was virtually cell free (Table 2).

To investigate the influence of repeated freezing and thawing on the residual cell levels, we carried out freezing experiments with Autopheresis-C source plasma and in-line filtered plasmas type A and type C. We found that the freezing process only had a minor influence on the platelet content (Figure 4). In addition, the low leucocyte levels found (Figure 4) in the two in-line filtered plasma types before freezing indicated that the in-line filtration procedures are highly effective in reducing the leucocyte content of whole blood. Erythrocytes were destroyed by the freezing and thawing process, resulting in the detection of many erythrocyte "ghosts" after the process (Figure 4). Interestingly, the measurement LDH, a general marker for cell damage [19], demonstrated no differences between the plasma types (Table 3). Thus, the in-line filtration process seemed to have no influence on this variable.

Platelet-associated proteins in different plasma types

The residual platelet content in the plasma samples suggested that platelets were affected by the in-line filtration procedure. This prompted us to measure the content of PF4 and bTG, which are probably indicative for the level of platelet damage, in the different plasma preparations. By exerting stress on platelets by freeze-drying, which is known to disrupt cells, we demonstrated a linear relation between bTG and PF4 concentrations and the number of damaged platelets (Figure 6). In particular, a harsh lyophilisation procedure released PF4 and bTG from platelets, while the process of freezing and thawing alone was not responsible for cell damage (Figure 6).

Figure 5 and Table 3 summarize our results obtained for PF4 and bTG for the frozen and thawed samples (Table 3 also shows the LDH data). Higher values were obtained for the in-line filtered plasma types than for plasma recovered by the classic process, confirming our hypothesis that platelets are disrupted by the filtration process. The lowest PF4 and bTG concentrations were found for source plasma prepared with the Autopheresis-C system. Haemonetics source plasma had much higher values, suggesting that the centrifugation caused platelet damage by shear stress. Values obtained for source plasma (Autopheresis-C) and in-line filtered plasma type C were hardly affected by the freezing and thawing process (Figure 6), which seemed to be essential for the release of platelet-associated proteins

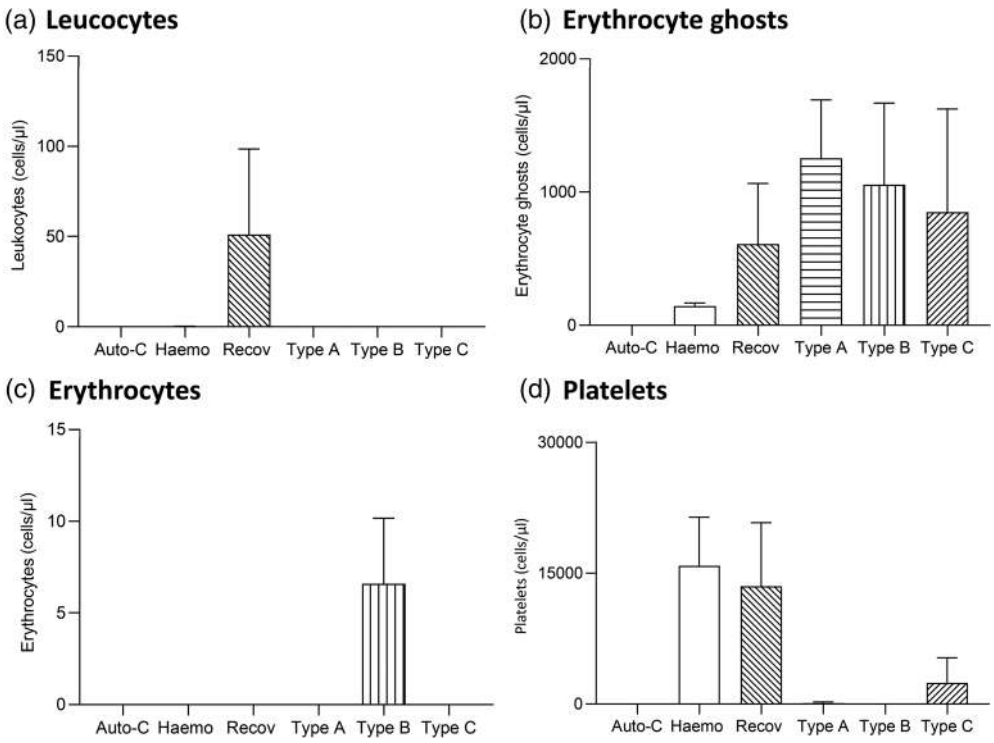


FIGURE 3 Residual cell count in different plasma types. The mean cell counts for leucocytes (a) and erythrocytes (c), while (b) shows the means for erythrocytes (ghosts) and (d) for platelets. The following numbers of single plasma samples were averaged: Source plasma (Autopheresis C), $n = 10$; source plasma (Haemonetics), $n = 10$; recovered plasma (classic), $n = 12$; in-line filtered plasma type A, $n = 10$; in-line filtered plasma type B, $n = 10$; in-line filtered plasma type C, $n = 26$

TABLE 2 Residual cell content in different plasma types

	Cells/µl (mean ± SD)			
Plasma Type	**Leucocytes**	**Erythrocytes[a]**	**Erythrocytes[b]**	**Platelets**
Source plasma (Auto-C), $n = 10$	0	0	0	8 ± 3
Source plasma (Haemonetics), $n = 10$	0.18 ± 0.14	0	143 ± 25	15 860 ± 5590
Recovered plasma (classic), $n = 12$	51 ± 48	0	610 ± 455	13 503 ± 7297
In-line filtered plasma type A, $n = 10$	0.006 ± 0.013	0	1255 ± 436	100 ± 127
In-line filtered plasma type B, $n = 10$	0	7 ± 4	1056 ± 613	3 ± 2
In-line filtered plasma type C, $n = 26$	0	0	849 ± 775	2447 ± 2861
ANOVA results ($\alpha = 0.05$)[c]	$p < 0.0001$	Not done	$p = 0.0247$	$p = < 0.0001$

Note: Remarks: Means ± standard deviations are shown. The abbreviation n.d. stands for not detected.
[a]The column shows the erythrocyte count with normal phenotype.
[b]The number of erythrocyte "ghosts" is shown.
[c]One-way ANOVA was performed followed by Tukey's multiple comparisons test using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California. Results obtained for the individual comparison of groups with the Tukey's multiple comparison test are shown in Supplemental Material.

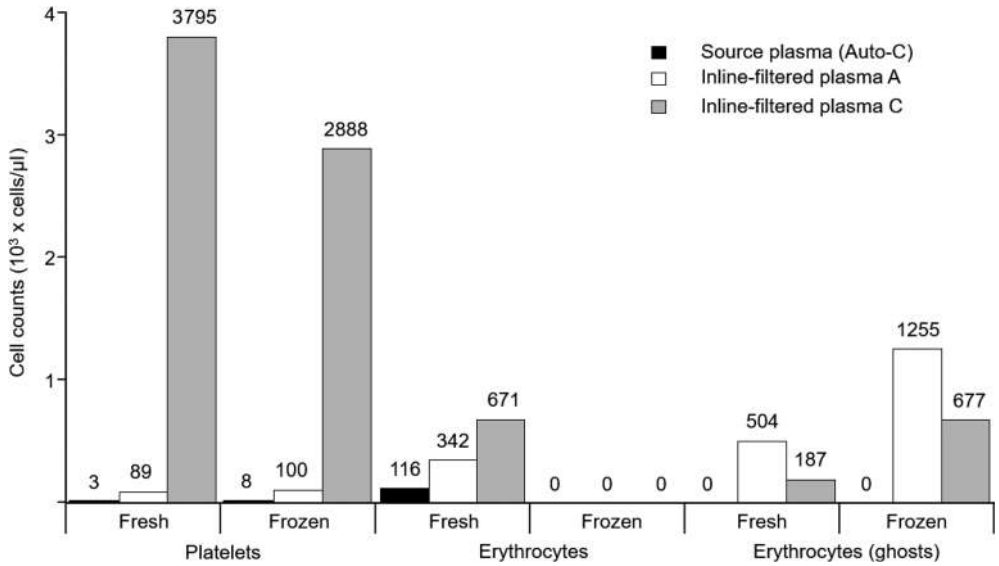


FIGURE 4 Influence of freezing-thawing on residual cell content in different plasma sources. Erythrocytes, erythrocytes (ghosts) and platelets were measured in fresh and frozen samples of source plasma (Autopheresis C) and of in-line filtered plasma types A and C

TABLE 3 Cell-associated proteins in different plasma types

Plasma Type	**LDH (U/l)**	**PF4 (IU/ml)**	**bTG (IU/ml)**
Source plasma (Auto-C), $n = 10$	113 ± 20	161 ± 85	176 ± 62
Source plasma (Haemonetics), $n = 10$	142 ± 28	630 ± 210	2145 ± 804
Recovered plasma (classic), $n = 12$	113 ± 20	239 ± 95	308 ± 116
In-line filtered plasma type A, $n = 10$	139 ± 30	1680 ± 1093	3199 ± 1572
In-line filtered plasma type B, $n = 10$	111 ± 21	420 ± 371	1129 ± 562
In-line filtered plasma type C, $n = 26$	114 ± 26	805 ± 488	1321 ± 1095
ANOVA ($\alpha = 0.05$)[a]	$p = 0.0053$	$p < 0.0001$	$p < 0.0001$
Normal physiological range	<240	0–5	10–50

Note: Remarks: Means ± standard deviations (SD) are shown.
[a]One-way ANOVA followed was performed by Tukey's multiple comparisons test using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California. Results obtained for the individual comparison of groups with the Tukey's multiple comparison test are shown in Supplemental Material.

in in-line filtered plasma type A, indicating a different mechanism of platelet disruption than for in-line filtered plasma type C (Figure 6).

Platelet function analysis with PFA 100

We investigated the influence of whole blood filtration with in-line filter type A to further confirm our hypothesis that platelets are affected by whole blood in-line filtration. We measured whole blood mean closure times of 163 s with collagen-EPI cartridges and of 95 s with collagen-ADP cartridges. These closure times were within the published reference ranges of 98–185 s for collagen-EPI cartridges and 77–133 s for collagen-ADP cartridges [42]. After filtration through the appropriate filter device, no definite closure times (>300 s) could be measured for any of the samples with either of the cartridges. This reflects the reduction in content of intact platelets resulting from the filtration process. The content, as determined by the whole blood cell counter, went down from 290,000 to 30,000 cells/µl.

Determination of blood cell-associated marker proteins in manufacturing plasma pools

The study described above was performed with a limited number of single donor plasmas and showed the variability of platelet-associated proteins PF4 and bTG in different plasma types used for fractionation. Therefore, it was of special interest to investigate if these results could be verified for manufacturing plasma pools used in the plasma

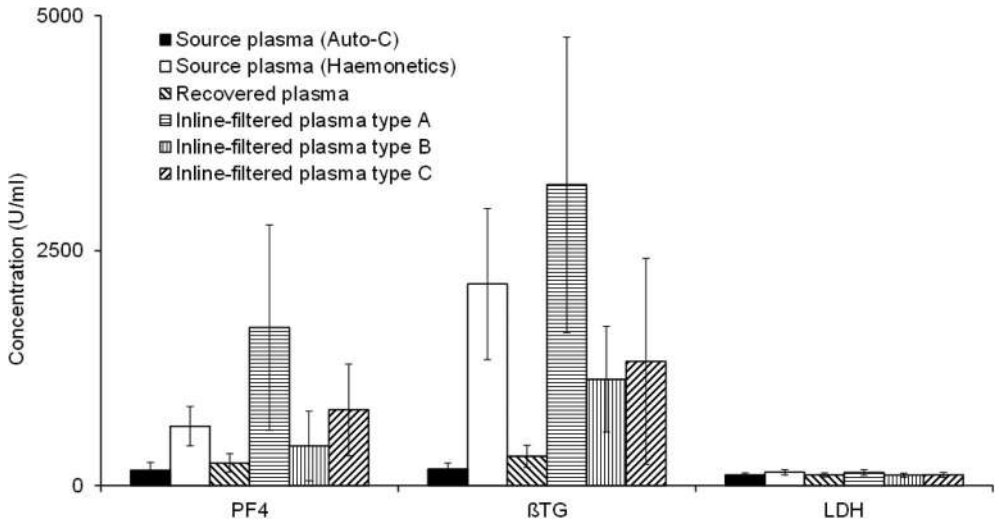


FIGURE 5 Cell-associated protein levels in different plasma types. The mean concentrations of the cell-associated proteins PF4, bTG and LDH are shown. The following numbers of single plasma samples were averaged: Source plasma (Autopheresis C), $n = 10$; source plasma (Haemonetics), $n = 10$; recovered plasma (classic), $n = 12$; in-line filtered plasma type A, $n = 10$; in-line filtered plasma type B, $n = 10$; in-line filtered plasma type C, $n = 26$

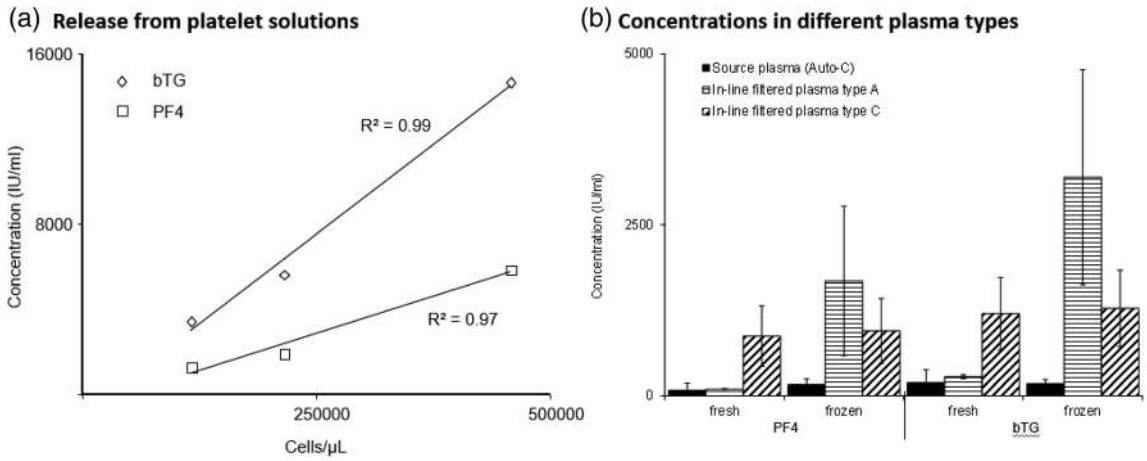


FIGURE 6 Release of PF4 and bTG after freezing and thawing. (a) PF4 and bTG levels in platelet suspensions, adjusted to 125,000, 250,000 and 500,000 cells/µl. PF4 and bTG are released by a procedure including deep freezing at −70°C, lyophilisation and reconstitution of the samples with water. (b) The concentrations of PF4 and bTG in different plasma types

TABLE 4 PMN elastase and PF4 levels in manufacturing plasma pools

Plasma Type	PMN Elastase (μg/ml)	PF4 (IU/ml)
Source plasma (Auto-C), $n = 36$	69 ± 29 (29–136)	114 ± 143 (8–508)
Recovered plasma, $n = 24$	123 ± 87 (32–453)	369 ± 336 (22–1026)
In-line filtered plasma, $n = 22$	329 ± 145 (125–677)	1023 ± 256 (600–1656)
ANOVA ($\alpha = 0.05$)[a]	$p < 0.0001$	$p < 0.0001$
Tukey's multiple comparison test: Source vs. recovered	$p = 0.0651$	$p = 0.0005$
Tukey's multiple comparison test: Source vs. in-line filtered	$p < 0.0001$	$p < 0.0001$
Tukey's multiple comparison test: Recovered vs. in-line filtered	$p < 0.0001$	$p < 0.0001$

Note: Remarks: Means ± SD values are shown. The numbers in brackets give the minimum and maximum values determined. Plasma pools of source and recovered/in-line filtered plasma correspond to 2000 to 2800 and 4200 to 6600 single plasma units, respectively.
[a]One-way ANOVA was performed followed by Tukey's multiple comparisons test using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California.

fractionation process. Thus, we measured PF4 as a selected variable in different plasma pools of source plasma (Autopheresis-C), recovered plasma and in-line filtered plasma consisting of 2000 to 6600 single plasma units, each. As we demonstrated that platelets were affected by in-line filtration of whole blood, we decided to also determine the levels of elastase from polymorphonuclear (PMN) leucocytes as a marker for testing the integrity of leucocytes. Our results are summarized in Table 4. Like the results above, source plasma pools were shown to have very low mean PF4 levels (114 IU/ml). Higher mean values were found for recovered plasma (369 IU/ml) and in-line filtered plasma (1023 IU/ml). A similar relation between plasma type and marker protein level was found for elastase, determined and present as an inactive elastase-alpha1-antitrypsin complex. Thus, we measured mean concentrations of 69, 123, and 329 μg/ml for source, recovered and in-line filtered plasma pools, respectively. The PMN elastase values for in-line filtered plasma were substantially higher than for recovered plasma. This is a strong indicator for the fact that leucocytes are not only depleted from whole blood by the in-line filtration procedure but also that the filtration process obviously affects the integrity of the leucocytes leading to a release of PMN elastase. PMN elastase activity, however, is immediately neutralized by effective complex formation with alpha1 antitrypsin.

DISCUSSION

Currently safety and quality of plasma for fractionation focus on pathogen safety issues, while protein-specific variables (e.g. FVIII levels) in plasma for fractionation are not considered to be related to the basic need for assuring the quality and safety of plasma. In his review [43], Farrugia proposed that such variables should be restricted to arrangements between the supplier of plasma and manufacturer of its products. However, variables and standards need to be set for plasma for fractionation, preferentially on a global level, for regulatory authorities to assess the essential safety features, particularly those contributing to viral safety, independently from issues centred around the need for specific products, such as coagulation factors [6, 10–12, 23]. While for obvious reasons there are numerous publications on pathogen safety, studies on plasma quality assessed by biochemical variables are relatively rare. Consequently, in a comprehensive study, clotting factors and several critical proteins were measured and compared in plasma obtained after whole-blood filtrations with different filters and non-filtered plasma [44]. In our comparative study, we chose six different plasma types used as starting plasma for fractionation: one source plasma prepared with the Autopheresis-C and another with the Haemonetics PCS-2 system, plasma recovered by the classic method and three types of in-line filtered plasmas prepared from whole blood that had been subjected to different filter systems to reduce the leucocyte load.

We selected several biochemical and cellular markers to characterize the plasma types. Our tests for biochemical markers comprised protein content, coagulation factors FVIII and VWF, prothrombin fragment (F1 + 2) and TAT, two activation markers of coagulation [45], FXIIa, a marker of contact activation [46, 47], and complement factor C3a, a marker of complement activation [48]. In addition, we determined the residual cell content (platelets, leucocytes, erythrocytes, and erythrocyte "ghosts") by FACS analysis [37] and measured different cell-specific markers: LDH as a general marker of cell damage [19] and the platelet-associated proteins [49] PF4 and bTG, which are constituents of alpha granules [50] and are known as heparin-neutralizing proteins [51]. In another series of analyses, we measured PF4 and PMN elastase in plasma pools used for fractionation, representing more than 10,000 single plasma units.

By measuring these biochemical and cellular markers, we found that there are substantial differences between plasma types in complement activation, TAT values and, to a minor extent, FXIIa concentrations. Our results for complement activation in source plasma prepared by apheresis technology confirmed previous reports of complement activation on membranes via the alternative pathway [44, 52–54]. The most striking differences between the plasma types we investigated were, however, in the residual cell content and cell-associated proteins. This confirms previous studies reporting that source plasma prepared by the Autopheresis-C system is virtually cell-free [55].

Our results also show that leucocyte reduction of whole blood is highly effective, but the depletion procedure is accompanied by the

release of PMN elastase. This finding contrasts with an earlier study where no increase in PMN elastase levels was found after in-line filtration of whole blood by use of different filter systems [56]. However, our study confirms a more recent finding of strong neutrophil and complement activation, which depended on the type of filter and whole-blood storage conditions [44]. The authors of this study concluded that despite the considerable elastase release by whole-blood filtration, as detected by the increase in elastase-antitrypsin complexes, the plasma showed no sign of proteinase activity due to a sufficient inhibitory antitrypsin activity of a healthy donor. Additionally, our data demonstrate that the integrity of leucocytes is obviously strongly affected by the filtration process with not only activation of neutrophil and the subsequent release of elastase during the filtration process, but also a complete disruption of cells. Several studies investigated how prions, which are believed to cause vCJD, can be eliminated during the manufacture of pharmaceutical products derived from human blood [57, 58]. There is evidence to indicate that any infectivity present may be reduced during these processes [59]. The implication of lymphoid tissues and lymphocytes in the peripheral pathogenesis of prion disease was the rationale for introducing leucodepletion of blood donations to reduce possible vCJD transmission by blood components [60]. However, this practice, although implemented in many countries and mandatory in some, was questioned by some authors who believed at best it was only hypothetically suitable for preventing the transmission of vCJD and there had already been a vague discussion concerning potential risks of leucodepletion such as increased leakages, which had not been investigated empirically [61, 62]. As our results indicate that the in-line filtration process destroys cells instead of or in addition to removing them, it seems questionable if any benefit can be gained by removing leucocytes, which would also remove leucocyte-associated proteins and pathogens including vCJD. The impact of in-line filtration on blood cells is furthermore reflected by both the platelet counts in whole blood and the functional characterization of platelets in the PFA-100 system. Intact platelets were reduced substantially to about 10% of the value before in-line filtration and the reduced number of functionally active platelets was ineffective to obtain full occlusion of a collagen-coated capillary. However, our results show that the passage of blood through leucocyte reduction filters not only removes but disrupts the remaining platelets and promotes the release of the platelet-associated proteins PF4 and bTG. Similarly, an increase in bTG concentrations has been observed after in-line filtration [63]. In our study we used an additional platelet protein marker, PF4, which is an alpha-granule protein [50] that originates from disrupted platelets. In contrast to our findings, another group found no increase in bTG values by in-line filtration of whole blood [56], suggesting differences in filtration conditions might play a role in this process. Despite the effects observed on cellular blood components and marker proteins derived from them, most of the plasma separation methods did not affect the proteins of the VWF complex. This is remarkable because VWF with a maximum molecular size of ≥20 million Dalton [64] is not only the largest circulating soluble plasma protein, and consequently difficult to filter [65], but is also subject to specific shear-dependent degradation by ADAMTS13, a metalloprotease [66–69]. Nevertheless, the largest VWF multimers were not lost due to the separation process. There was one exemption: The PCS-2 system, which showed a somewhat lower content of the high molecular weight VWF multimers. There is currently no explanation for this and to find one further detailed analysis would be required. However, our result is in-line with that found in a previous study, where the VWF content of normal plasma pools was compared with plasma obtained by five different apheresis procedures and where VWF:Ag, VWF:RCo and 11–15 VWF multimers were well preserved in all plasma units from each of the five apheresis procedures [70]. Nevertheless, the highest molecular weight forms of VWF are missing in all plasma products as a consequence of co-purification of VWF with its cleaving protease ADAMTS13, which degrades VWF in plasma starting during apheresis and continuing throughout the fractionation process. In addition, our study shows that the filtration process of whole blood can lead to activation of the complement system, as indicated by an increase in C3a concentrations, regardless of whether the process is performed in-line or by a frequently used filtration-based plasmapheresis system [54].

So far, most studies on starting plasmas for fractionation have concentrated on the freezing process and its possible influence on the levels of coagulation factors [41, 71, 72]. Only a few have investigated the influence different plasma types have on the manufacturing of therapeutic coagulation proteins. With respect to the high sensitivity of the FVIII molecule to proteolysis, the measure of the coagulation activation marker fibrinopeptide A was introduced as a criterion for determining the quality of plasma as a source of factor VIII [73]. Some data suggest that FVIII degradation products present in plasma of different qualities, in addition to other factors, might lead to increased immunogenicity of FVIII concentrates [74], and molecular modifications have been detected in factor VIII concentrates produced from different plasma pools [75]. Other investigators described the effects of the plasma collection systems and processing variables on the quality of factor IX concentrate and found an increased thrombogenic potential in plasma prepared by centrifugation [7]. Furthermore, different qualities of plasma for fractionation were found in one report, where the investigators recommended avoidance of platelet contamination and platelet activation [8]. These publications underline the importance of minimizing the residual cell content, especially platelets, containing negatively charged phospholipids, which can serve as procoagulatory surfaces [76]. If the residual platelet content is not minimized, unwanted side effects such as the promotion of activation phenomena [77] or thrombogenicity problems [7, 8] may occur. In addition, it should be emphasized that the anticoagulant efficacy of heparin added in some manufacturing processes might be impacted by PF4. However, as the neutralization of 1 IU heparin requires about 16,000 IU PF4 [78], this seems to be an important but only theoretical concern, as such high PF4 levels were not observed in our study.

In summary, our findings indicate that besides several specific tests, the platelet surrogate markers bTG and PF4 might be valuable for comparing plasma sources, especially for evaluating new or modified devices for plasma collection and for monitoring biochemical plasma quality.

ACKNOWLEDGEMENTS

We thank Eveline Gruber, Irmgard Billwein, Elisabeth Gangl, and Andrea Engelmaier for their technical assistance, Elise Langdon-Neuner for editorial assistance, and Manuela Leibrecht for assistance with the manuscript.

J.S. designed the study and wrote the manuscript. A.W. performed the analysis, created the tables and figures, and designed the manuscript. C.B. evaluated the data and reviewed the manuscript. P.L.T. guided the research, evaluated the data, and reviewed and edited the manuscript.

CONFLICT OF INTEREST

The work is sponsored by Baxalta Innovations GmbH, part of Takeda. At the time of the study, all authors were full-time employees of Baxalta Innovations GmbH and Baxter AG, respectively.

ORCID

Peter L. Turecek https://orcid.org/0000-0002-6161-1556

REFERENCES

1. Brodniewicz-Proba T. Human plasma fractionation and the impact use of new technologies on the use and quality of plasma-derived products. Blood Rev. 1991;5:245–57.
2. Vandersande J. Current approaches to the preparation of plasma fractions. Biotechnology. 1991;19:165–76.
3. Burckhardt JJ. Assessment of needs for plasma for fractionation in Europe. Biologicals. 1999;27:337–41.
4. Bertolini J, Hayes T. Plasma for fractionation. In: Bertolini J, Goss N, Curling J, editors. Production of plasma proteins for therapeutic use. first ed. New York: John Wiley & Sons Inc.; 2013.
5. Schuzki T, Seidel K, Storch H, et al. A prospective multicentre study on the safety of long-term intensive plasmapheresis in donors (SIPLA). Vox Sang. 2006;91:162–73.
6. Burnouf T, Radosevich M. Reducing the risk of infection from plasma products: Specific preventative strategies. Blood Rev. 2001;14:94–110.
7. Farrugia A, Spiers D, Young I, et al. Effects of plasma collection systems and processing parameters on the quality of factor IX concentrate. Vox Sang. 1989;57:4–9.
8. Smith JK. Quality of plasma for fractionation-does it matter? Transfus Sci. 1994;15:343–50.
9. Heiden M, Seitz R. Quality of therapeutic plasma – Requirements for marketing authorization. Thromb Res. 2002;107(Suppl 1):S47–51.
10. Morgenthaler JJ. Securing viral safety for plasma derivatives. Transfus Med Rev. 2001;15:224–33.
11. Sherman LA. NAT and blood safety. Transfusion. 2001;41:304–5.
12. Council of Europe. Guide to the preparation use and quality assurance of blood components, 20th edition. Council of Europe Publishing 2020.
13. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Food and Drugs, 2020, part 640, "Additional standards for human blood and blood products", subpart D - plasma, 640.30
14. Coffe C, Coudurier N, Levy G, et al. Comparative study of four new automated donor plasmapheresis systems. Plasma Ther Transfus Technol. 1986;7:49–55.
15. Nydegger UE, Pflugshaupt R. Quality control of blood plasma harvested by the flat-sheet filter system Autopheresis-C. Plasma Ther Transfus Technol. 1986;7:57–9.
16. Burkhardt T, Rothe R, Moog R. Immunoglobulin levels during collection of large volume plasma for fractionation. Transfus Apher Sci. 2017;56:417–20.
17. Richter E, Mollenkopf D, Kerowgan M, et al. Improved plasma quality by Haemonetics PCS-2 using the centered core blow molded bowl. Beitr Infusionsther Transfusionsmed. 1996;33:72–5.
18. Riggert J, Schwartz DWM, Wieding JU, et al. Prestorage inline filtration of whole blood for obtaining white cell-reduced blood components. Transfusion. 1997;37:1039–44.
19. Larsson S, Gulliksson H, Paunovic D. Evaluation of whole-blood WBC-reduction filter that saves platelets: in vitro studies. Transfusion. 2001;41:534–9.
20. Cardigan R, Sutherland J, Garwood M, et al. The effect of leucocyte depletion on the quality of fresh-frozen plasma. Br J Haematol. 2001;114:233–40.
21. Shinar E, Prober G, Yahalom V, et al. WBC filtration of whole blood after prolonged storage at ambient temperature by use of an in-line filter collection system. Transfusion. 2002;42:734–7.
22. Farrugia A. Risk of variant Creutzfeld-Jakob disease from factor concentrates: Current perspectives. Haemophilia. 2002;8:230–5.
23. Cerenakova L. Creutzfeldt-Jakob disease and blood transfusion: Safe or not safe? Transfusion. 2017;57:1851–4.
24. Waytes AT, Igel H, Zerlauth G, et al. A safer plasma supply from remunerated donors- "The Immuno Community Bio Resources experiment". Dev Biol Stand. 2000;102:37–51.
25. Legler TJ, Riggert J, Dove K, et al. Quantitative PCR for counting residual white blood cells in blood products. Transfus Sci. 1999;20:107–11.
26. Burnouf T, Kappelsberger C, Frank K, et al. Protein composition and activation markers in plasma collected by three apheresis procedure. Transfusion. 2003;43:1223–9.
27. Runkel S, Haubelt H, Hitzler W, et al. The quality of plasma collected by automated apheresis and of recovered plasma from leukodepleted whole blood. Transfusion. 2005;45:427–32.
28. Runkel S, Bach J, Haubelt H, et al. The impact of two whole blood inline filters on markers of coagulation, complement and cell activation. Vox Sang. 2005;88:17–21.
29. Siekmann J, Gruber E, Lagler I, et al. Analytical characterization of different plasma qualities used for fractionation. Ann Hematol. 2002;81(Suppl 1):A79.
30. Kießig ST, Krause K-P, Hellstern P. Comparison of source plasma quality in three different apheresis pools. Transfusion. 2012;52:2273–5.
31. Doumas BT, Bayse DD, Carter RJ, et al. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem. 1981;27:1642–50.
32. Rosen S. Assay of factor VIII:C with a chromogenic substrate. Scand J Haematol. 1984;33(Suppl 40):139–45.
33. Macfarlane DE, Stibbe J, Kirby EP, et al. A method for assaying von Willebrand factor (ristocetin cofactor). Thromb Diath Haemorrh. 1975;34:306–8.
34. Siekmann J, Turecek PL, Schwarz HP. The determination of von Willebrand factor activity by collagen binding assay. Haemophilia. 1998;4(Suppl 3):15–24.
35. Ruggeri ZM, Zimmerman TS. The complex multimeric composition of factor VIII/von Willebrand factor. Blood. 1981;57:1140–3.
36. Turecek PL, Gritsch H, Pichler L, et al. In vivo characterization of recombinant von Willebrand factor in dogs with von Willebrand disease. Blood. 1997;90:3555–67.
37. Pichler J, Printz D, Scharner D, et al. Improved flow cytometric method to enumerate residual cells: minimal linear detection limits for platelets, erythrocytes, and leucocytes. Cytometry. 2002;50:231–7.
38. Kundu SK, Heilmann EJ, Sio R, et al. Description of an in vitro platelet function analyzer PFA-100. Semin Thromb Hemost. 1995;21(Suppl 2):106–12.
39. Favaloro EJ. Clinical utility of closure times using the platelet function analyzer-100/200. Am J Haematol. 2017;92:398–404.
40. Shanbrom E, Lundak R, Walford RL. Long-term plasmapheresis: Effects on specific plasma proteins. Transfusion. 1972;12:162–7.

41. Hellstern P, Bach J, Haubelt H, et al. The impact of the intensity of serial automated plasmapheresis and the speed of deep-freezing on the quality of plasma. Transfusion. 2001;41:1601–5.
42. Mammen EF, Alsahameeri RS, Comp PC. Preliminary data from a field trial of the PFA-100 system. Semin Thromb Hemost. 1995;21 (Suppl 2):113–21.
43. Farrugia A. Plasma for fractionation: Safety and quality issues. Haemophilia. 2004;10:334–40.
44. Heiden M, Salge U, Henschler R, et al. Plasma quality after whole-blood filtration depends on storage temperature and filter type. Transfus Med. 2004;14:297–304.
45. Bauer KA. Activation markers of coagulation. Clin Haematol. 1999; 12:387–406.
46. Chandra S, Wickerhauser M. Contact factors are responsible for the thrombogenicity of prothrombin complex concentrates. Thromb Res. 1979;14:189–98.
47. Boisclair MD, Philippou H, Lane DA. Thrombogenic mechanisms in the human: Fresh insights obtained by immunodiagnostics studies of coagulation markers. Blood Coagul Fibrinolysis. 1993;4:1007–21.
48. Morgan BP. The complement system: An overview. Methods Mol Biol. 2000;50:1–13.
49. Pepper DS, Prowse CV. Beta-thromboglobulin and platelet factor 4. In: Jesperson J, Bertina RM, Haverkate F, editors. ECAT assay procedures. Dordrecht NL: Kluver Academic Publishers; 1990. p. 21–34.
50. Harrison P, Cramer EM. Platelet α-granules. Blood Rev. 1993;7: 52–62.
51. Maccarana M, Lindahl U. Mode of interaction between platelet factor 4 and heparin. Glycobiology. 1993;3:271–7.
52. Svehag SE. Adverse effects of clinical intervention on the complement system. Complement Inflamm. 1991;8:359–69.
53. Aeschbacher B, Haeberli A, Nydegger UE. Donor safety and plasma quality in automated plasmapheresis. Comparison of two filter materials. Vox Sang. 1989;57:104–11.
54. Schooneman F, Marchand M, Vigneron C, et al. Determination of C3a and C5a in hemapheresis procedures. Ann Med Interne (Paris). 1990;141:597–9.
55. Neumeyer HF, Quentin SH, Wieding JU. Comparative analysis of various plasmapheresis methods-modern procedures of mechanical plasma collection compared with each other and with manual bag centrifugation procedures. Beitr Infusionsther. 1993;29:163–89.
56. Prowse CV, Hornsey VS, Drummond O, et al. Preliminary assessment of whole-blood, red-cell and platelet-leucodepleting filters for possible induction of prion release fragmentation during room temperature processing. Br J Haematol. 1999;106:240–7.
57. Reichl HE, Foster PR, Welch AG, et al. Studies on the removal of a bovine spongiform encephalopathy-derived agent by processes used in the manufacture of human immunoglobulin. Vox Sang. 2002;83: 137–45.
58. Stenland CJ, Lee DC, Brown P, et al. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. Transfusion. 2002;42: 1497–500.
59. Ironside JW, Head MW. Variant Creutzfeldt-Jakob disease and its transmission by blood. J Thromb Haemost. 2003;1:1479–86.
60. Mc Gregor IR, Prowse CV. Impacts and concerns for vCJD in blood transfusion: Current status. Curr Mol Med. 2004;4:361–73.
61. St Romaine C, Hazlehurst G, Jewell AP. Leucodepletion for transmissible spongiform encephalopathies. Br J Biomed Sci. 2004;61:48–54.
62. Karger R, Kretschmer V. Inline-filtration. Transfus Apheresis Sci. 2002;27:137–52.
63. Riedner C, Heim MU, Mempel W, et al. Possibility to improve preservation of whole blood by leucocyte-depletion before storage. Vox Sang. 1990;59:78–82.
64. Sadler JE. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem. 1998;67:395–424.
65. Favaloro EJ, Mohammed A, Coombs R, et al. Filtered plasma as a potential cause of clinical misdiagnosis: Inappropriate testing in a haematology laboratory. Br J Biomed Sci. 1995;52:243–8.
66. Furlan M, Robles R, Lämmle B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood. 1996;87:4223–43.
67. Zheng X, Chung D, Takayama TK, et al. Structure of von Willebrand factor cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem. 2001;276: 41059–63.
68. Plaimauer B, Zimmermann K, Völkel D, et al. Cloning, expression, and functional characterization of the von Willebrand factor–cleaving protease (ADAMTS13). Blood. 2002;100:3626–32.
69. Crawley JTB, de Groot R, Xiang Y, et al. Unraveling the scissile bond: How ADAMTS13 recognizes and cleaves von Willebrand factor. Blood. 2011;118:3212–21.
70. Burnouf T, Caron C, Burkhardt T, et al. Content and functional activity of von Willebrand factor in apheresis plasma. Vox Sang. 2004;87: 27–33.
71. Carlebjoerk G, Blombäck M. Freezing of plasma to obtain better yield of factor VIII:C. Scand J Haematol. 1984;33(Suppl 40):127–8.
72. Farrugia A, Prowse C. Studies on the procurement of blood coagulation factor VIII: Effects of plasma freezing rate and storage conditions on cryoprecipitate quality. J Clin Pathol. 1985;38: 433–7.
73. Pflugshaupt R, Kurt G. FPA content – A criterion of quality for plasma as factor VIII source. Vox Sang. 1983;45:224–32.
74. Josic D, Buchacher A, Kannicht C, et al. Degradation products of factor VIII which can lead to increased immunogenicity. Vox Sang. 1999;77(Suppl 1):90–9.
75. Saenko E, Josic D, Stadler M, et al. Molecular modifications in factor VIII concentrates produced from different plasma pools. Thromb Res. 2001;101:501–11.
76. Bevers EM, Comfurius P, Zwaal RFA. Platelet procoagulant activity: Physiological significance and mechanisms of exposure. Blood Rev. 1991;5:146–54.
77. Mannucci PM. Mechanisms, markers and management of coagulation activation. Br Med Bull. 1994;50:851–70.
78. Michalski R, Lane DA, Pepper DS, et al. Neutralization of heparin in plasma by platelet factor 4 and protamine sulphate. Br J Haematol. 1978;38:561–71.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Siekmann J, Weber A, Bauer C, Turecek PL. Biochemical and cellular markers differentiate recovered, in-line filtered plasma, and plasma obtained by apheresis methods. Vox Sang. 2022;117:27–38.

Received: 11 December 2020 | Revised: 7 April 2021 | Accepted: 14 April 2021

DOI: 10.1111/vox.13127

ORIGINAL PAPER

Vox Sanguinis ISBT International Society of Blood Transfusion

A comparison of the effect of X and gamma irradiation on red cell storage quality

Athinoula Meli[1] | Marie Anne Balanant[1] | Helen V. New[2,3] | Michelle Ray[4] | Elisa Allen[5] | Rebecca Cardigan[1,6] | Michael Wiltshire[1]

[1]Component Development Laboratory, NHS Blood and Transplant, Cambridge, UK

[2]Clinical Services Directorate, NHS Blood and Transplant, London, UK

[3]Department of Haematology, Imperial College London, London, UK

[4]Manufacturing Development Team, NHS Blood and Transplant, Oxford, UK

[5]Statistics and Clinical Studies, NHS Blood and Transplant, Bristol, UK

[6]Department of Haematology, University of Cambridge, Cambridge, UK

Correspondence: Athinoula Meli, Component Development Laboratory, NHS Blood and Transplant, Long Road, Cambridge, CB2 0PT, UK
Email: Athinoula.Meli@nhsbt.nhs.uk

Abstract

Background and Objectives: Irradiation of red cell components is indicated for recipients at risk of transfusion-associated graft vs. host disease. Current technologies available comprise of a gamma (γ) or an x source of radiation. The benefits of x vs. γ include non-radioactivity and hence no decay of the source. We aimed to compare the effect of the two technologies on red cell component storage quality post-irradiation.

Materials and Methods: Paired units of red cell concentrates (RCC), neonatal red cell splits (RCS), red cells for intra-uterine transfusion (IUT) or neonatal exchange transfusion (ExTx) were either γ- or x-irradiated. Units were sampled and tested for five storage parameters until the end of shelf life. Equivalence analysis of storage quality parameters was performed for pairs of the same components (RCC, RCS, IUT or ExTx) that were either γ- or x-irradiated.

Results: Nearly all component comparisons studied showed equivalence between γ and x irradiation for haemolysis, ATP, 2,3-DPG, potassium release and lactate production. The exceptions found that were deemed non-equivalent were higher haemolysis with x irradiation for ExTx, lower 2,3-DPG with x irradiation for RCS irradiated early and higher ATP with x irradiation for IUT. However, these differences were considered not clinically significant.

Conclusion: This study has demonstrated that a range of red cell components for use in different age groups are of acceptable quality following x irradiation, with only small differences deemed clinically insignificant in a few of the measured parameters.

KEYWORDS
irradiation, red cell concentrates, exchange transfusion, intra-uterine transfusion, Red cell splits

INTRODUCTION

Guidelines for blood transfusion services require the irradiation of red cell components indicated for recipients at risk of transfusion-associated graft vs. host disease (TA-GvHD) [1]. TA-GvHD is a rare condition where viable lymphocytes in transfused blood can proliferate in the recipient, particularly if they are severely immunocompromised, usually with fatal consequences [2]. TA-GvHD is successfully prevented with irradiation at a dose which renders lymphocytes non-viable, yet should not cause significant harm to other cell types. However, the *in vitro* quality of irradiated red cell components deteriorates faster during storage compared with non-irradiated red cells, and in mitigation irradiated red cell components have a reduced shelf life. In the UK, guidelines allow for standard red cells to be irradiated up to 14 days of storage, with a 14-day shelf life thereafter [3]. More flexibility is given with European guidelines, which state red cells may be

irradiated up to 28 days after collection with a 14-day shelf life thereafter (and no later than 28 days after collection) [4], while American guidelines allow irradiation at any time during storage (up to 42 days) with a 28 shelf life thereafter [5]. Guidelines on irradiation dose also differ in each country, and the irradiation source could be either gamma (γ) or X-ray. The Council of Europe [6], UK [1] and Australian and New Zealand [7] guidelines recommend that for red cells, platelets and granulocytes the minimum dose achieved in the irradiation field is 25 Gy, with no part receiving more than 50 Gy, based on lymphocyte proliferation assays and component quality studies [8]. The American and Japanese Blood services use a central dose of 25 Gy (minimum 15 Gy).

Gamma irradiation uses a radioactive source, which is subject to stringent security regulations. Also, due to the decay of the source, it requires regular recalibrations and expensive disposal of the equipment at end of life. In contrast, the X-ray sources are non-radioactive; hence, the above burdens are removed.

Although literature exists that evidences the equivalence of γ and X-rays in inactivating lymphocytes [9–11], there are limited data comparing the quality of red cell components post-γ or x irradiation. A previous study from our laboratory [12], as well as Janatpour K, 2005 [10], found no clinically significant difference between γ and x irradiation on red cell haemolysis and potassium leak over storage. In this study, we looked at additional components and metabolic parameters than previously studied to examine differences between γ and x irradiation more closely as x irradiation technology has developed in time and is being more widely implemented internationally. The current study was designed to ascertain whether red cell storage quality is adequate for a range of red cell components transfused to all age groups from foetuses to adult following x irradiation.

MATERIALS AND METHODS

Processing of components

This work was performed as two studies; in the first study, 40 Red Cell Concentrates (RCC) in Saline Adenine Glucose Mannitol (SAGM) were manufactured within the National Health Service Blood and Transplant (NHSBT) under standard procedures; that is leucocyte depleted (LD), manufactured and placed in cold storage (2–6ºC) within 27 h of venepuncture. Twenty of the units had been bled into FQE614B collection packs (Macopharma, Twickenham, UK), in which the whole blood is filtered prior to separation to remove platelets and leucocytes. These collection packs are used for the manufacture of plasma and red cells. The other 20 units were bled into LQT6283LB collection packs (Macopharma) in which the whole blood is separated into plasma, buffy coat and red cells, before the red cells are filtered. The FQE614B bags were centrifuged at 4200 ***g*** for 15 min at 22°C (Sorvall RC 3BP+; Kendro Laboratory Products, Bishops Stortford, UK), while the LQT6283LB bags were centrifuged at 3399 ***g*** for 10 min at 22°C (Sorvall RC 3BP+). For Neonatal Red Cell Splits (RCS), a full-sized RCC was split into six RCS by attaching an RCC to a set of sextuplet transfer packs (Paediatric red cell transfer packs – PRCB6 U; 600 ml; Macopharma) by sterile connection (TSCD; Terumo, Somerset, USA) in accordance with the manufacturer's instructions and allowed to drain evenly into the transfer packs by gravity.

In the second study, 40 whole bloods (WB; 475 ml ± 10%) collected into 66·5 ml of citrate phosphate dextrose anticoagulant using FQE614B collection packs (Macopharma) were leucocyte-depleted (LD) and then placed in cold storage (2–6ºC) within 12 h of venepuncture. On day one, 20 were manufactured into Neonatal Exchange Transfusion (ExTx) and 20 into intra-uterine transfusion (IUT) units. All WB were centrifuged at 4200 ***g*** for 15 min at 4°C (Sorvall RC 3BP+;). To produce ExTx, a volume of plasma was expressed (Luxomatic; Lmb; Schwaig; Germany) to achieve a haematocrit (Hct) of 50–55% (the NHSBT specification), while for IUT all the plasma was expressed using manual presses (Manual expresser; Baxter, IL, USA) into an intermediate transfer pack then 34–36 g of the plasma was added back to the red cells to achieve a Hct of 70–85%. The neonatal and foetal components were from standard donations, without NHSBT 'neonatal donor' specification, but this would not affect their characteristics for the purposes of this study.

Study design

Shown schematically in Fig. 1:

RCC and RCS study

Four SAGM RCC were pooled and split into four units on day one of storage, undertaken ten times to give ten units in each of the four study arms. Arms A and B were full-sized RCC, γ- or x-irradiated, respectively, on day 14 of storage. Arms Cγ and Cx were RCC split into RCS on day four and γ- or x-irradiated on day 14 of storage (late). Arms Dγ and Dx were RCC split into RCS on day four and γ- or x-irradiated on day five of storage (early). We irradiated on day 14 (late) as worst case according to UK guidelines, but also on day five (early) as most common practice in NHSBT.

IUT and ExTx study

Two IUT units were pooled and split into two arms E and F; similarly, two ExTx units were pooled and split into two arms G and H. This was undertaken ten times to give ten units in each arm. All units were tested for Hct on the day of production (day one) following pool and split. All units were γ- or x-irradiated on day four of storage as worst case. In the UK, ExTx and IUT have an overall five-day shelf life and are 100% irradiated with a 24-h shelf life post-irradiation. They are irradiated immediately before they are sent to hospitals.

For γ irradiation, the IBL437C model was used (CIS Bio International; Gif-sur-Yvette; France), and for x irradiation, the second

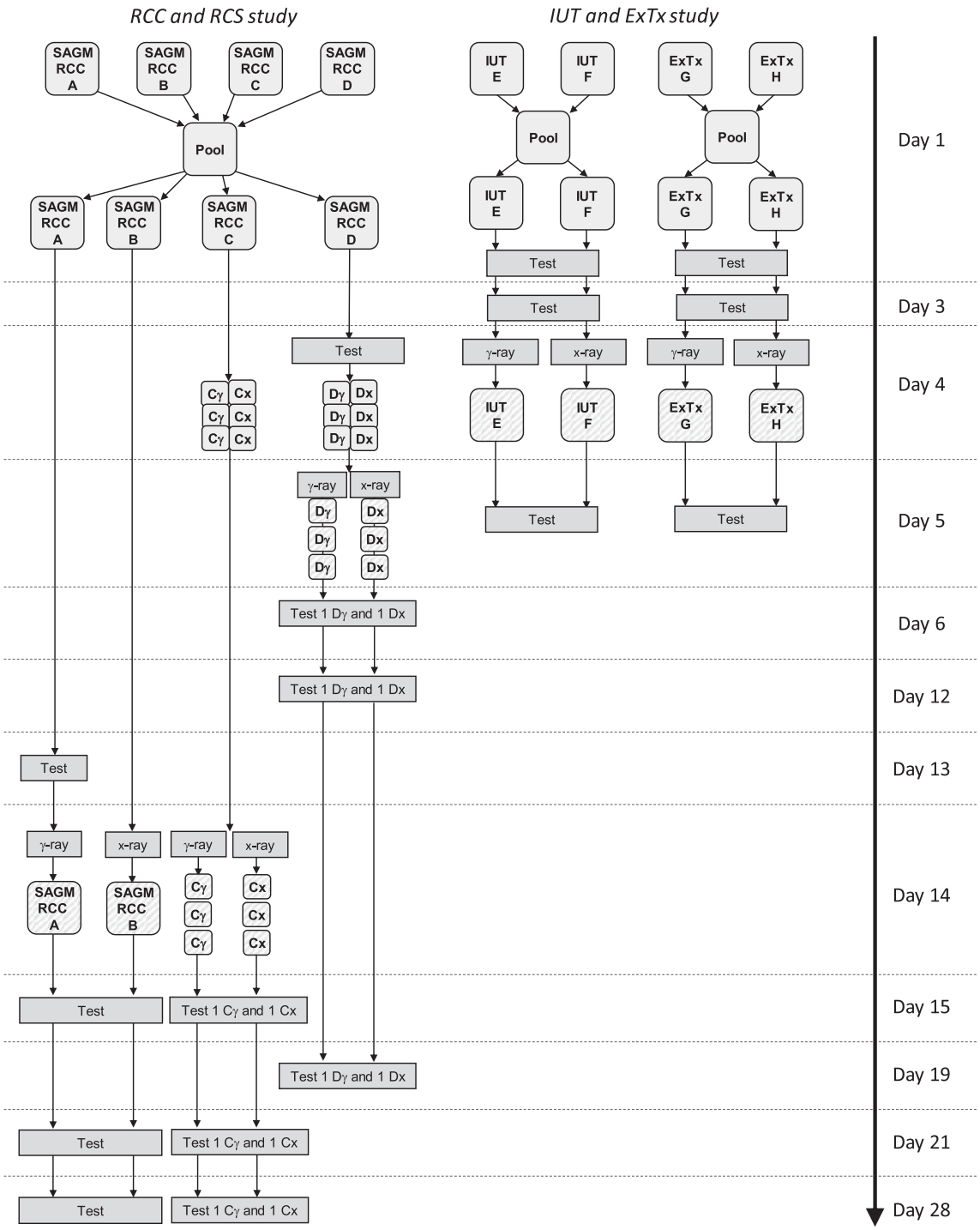


FIG. 1 Study design. Two studies were performed with four arms in each ($n = 10$ units in each arm). All units were stored at 2–6°C. *RCC and RCS* study: Arms A and B were full-sized RCC arms, γ- or x-irradiated on day 14 of storage. Arms C and D were RCC split into RCS on day four and γ or x-irradiated on day 14 of storage for arms Cγ and Cx, respectively, and on day five of storage for arms Dγ and Dx, respectively. *IUT and ExTx* study: Arms E and F were IUT and arms G and H were ExTx, γ- or x-irradiated on day four of storage. All arms in each study were tested a day post-irradiation and arms A, B, C and D were also tested day seven and day 14 post-irradiation. RCC, IUT and ExTx components were sampled on each testing day, while a single RCS was destructively tested on each testing day. SAGM, Saline Adenine Glucose Mannitol; RCC, Red cell concentrate; RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

generation RS3400 was used (Rad Source Technologies; GA, USA) both at a dose of ≥25 to ≤50 Gy. At the time of testing, dose mapping of the two irradiators was performed. Alanine dose mapping of the x irradiator, with all six canisters filled, at 310 seconds timer settings, measured the mean central dose at 35·6 Gy; minimum 27·5 Gy and maximum 46·6 Gy (3·5% uncertainty in measurement). Normalized measurements of the γ irradiator do map, at timer setting 9 min 14 seconds, with a 10 mm bottom spacer present, reported a central

dose at 33·2 Gy; minimum 26·7 Gy and a maximum 45·6 Gy (5% uncertainty in measurement).

Storage, sampling and testing of in vitro parameters

All units were stored at 4 ± 2°C. As units from arms A, B and C were paired, only arm A was tested a day before irradiation and represented the baseline for arms A, B and splits in arm C. Arm D was tested a day before irradiation and prior to splitting into RCS as baseline for arms Dγ and Dx. Arms E, F, G and H were all tested a day before irradiation. All arms A-H were tested a day post-irradiation, and arms A, B, C and D were also tested day seven and day 14 post-irradiation (Fig. 1).

RCC, IUT and ExTx were sampled on testing days using a sterile connected sample pouch (VSE0000A; Macopharma), while a single RCS pack from each arms Cγ, Cx, Dγ and Dx was destructively tested on each testing day.

Full blood counts (FBC) were performed using a Sysmex XN1000 haematology analyser (Sysmex Europe GmbH, Norderstedt, Germany). Red cell supernatants were prepared by double-centrifugation at 2000 ***g***

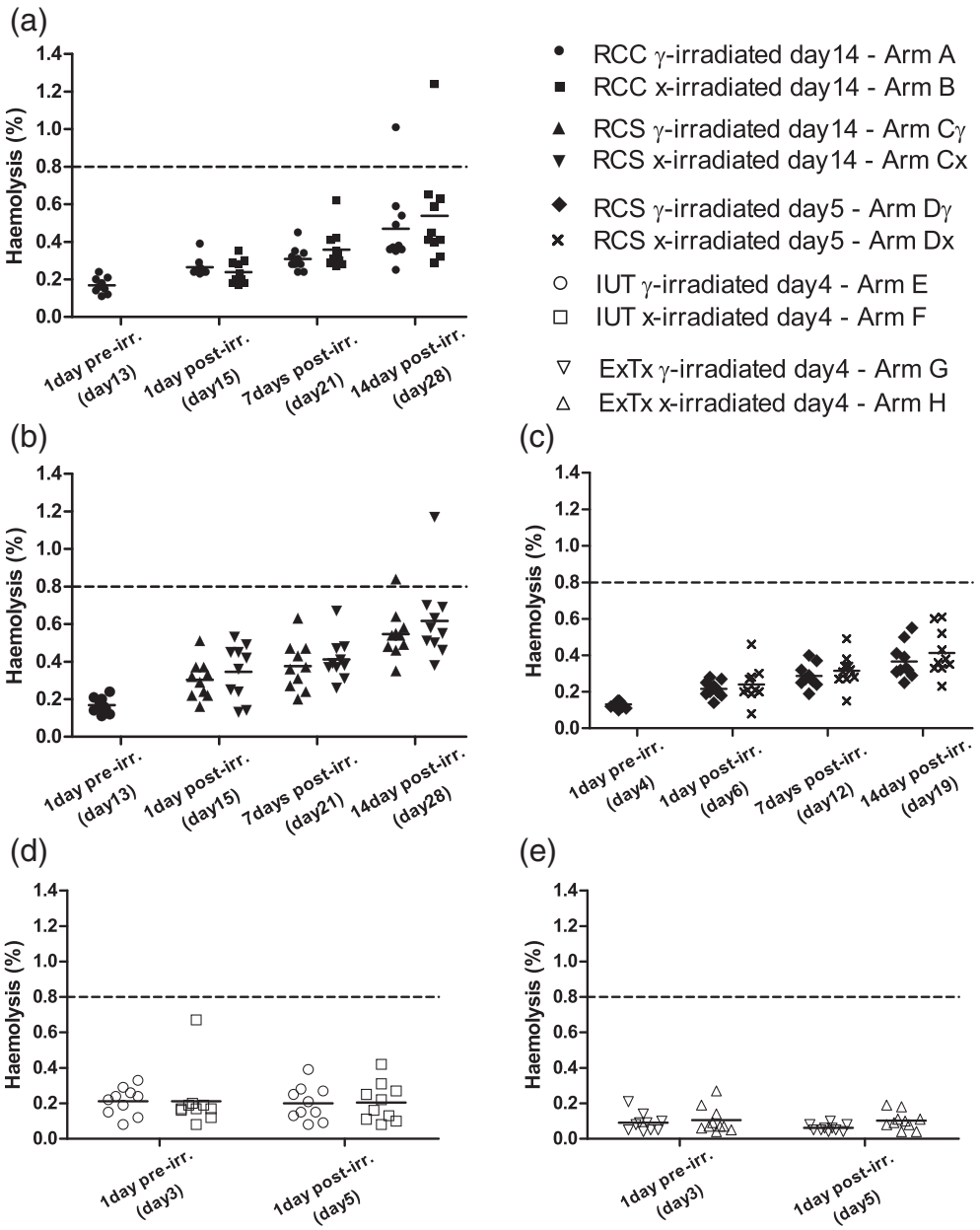


FIG. 2 Haemolysis. Shows haemolysis (%) levels for: a. the RCC comparison arm A vs. arm B; b. the RCS irradiated late comparison arm Cγ vs. arm Cx; c. the RCS irradiated early comparison arm Dγ vs. arm Dx; d. the IUT comparison arm E vs. arm F and e. the ExTx comparison arm G vs. arm H. Solid line shows mean value at each time point and within each arm ($n = 10$). Statistical analysis deemed γ and x irradiation equivalent for RCC, RCS and IUT but not for the ExTx comparison. Dotted line represents UK specification (>75% must be below this value). RCC, Red cell concentrate; RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

for 10 min (ALC PK130, DJB Labcare Ltd, Newport Pagnell, UK) and frozen at <−70°C. Supernatant haemoglobin (Hb) levels were determined using a direct spectrophotometric technique as previously described [13]. Supernatant potassium and lactate concentrations were measured using an Abbott Architect C16000 (Abbott, Maidenhead, Berkshire, UK).

For measurements of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG), red cell samples were deproteinized with perchloric acid as described previously [14] and stored below −70°C prior to testing. ATP was measured with a coupled enzyme method adapted from Lagerberg, JW et al. [15], while levels of 2,3-DPG were

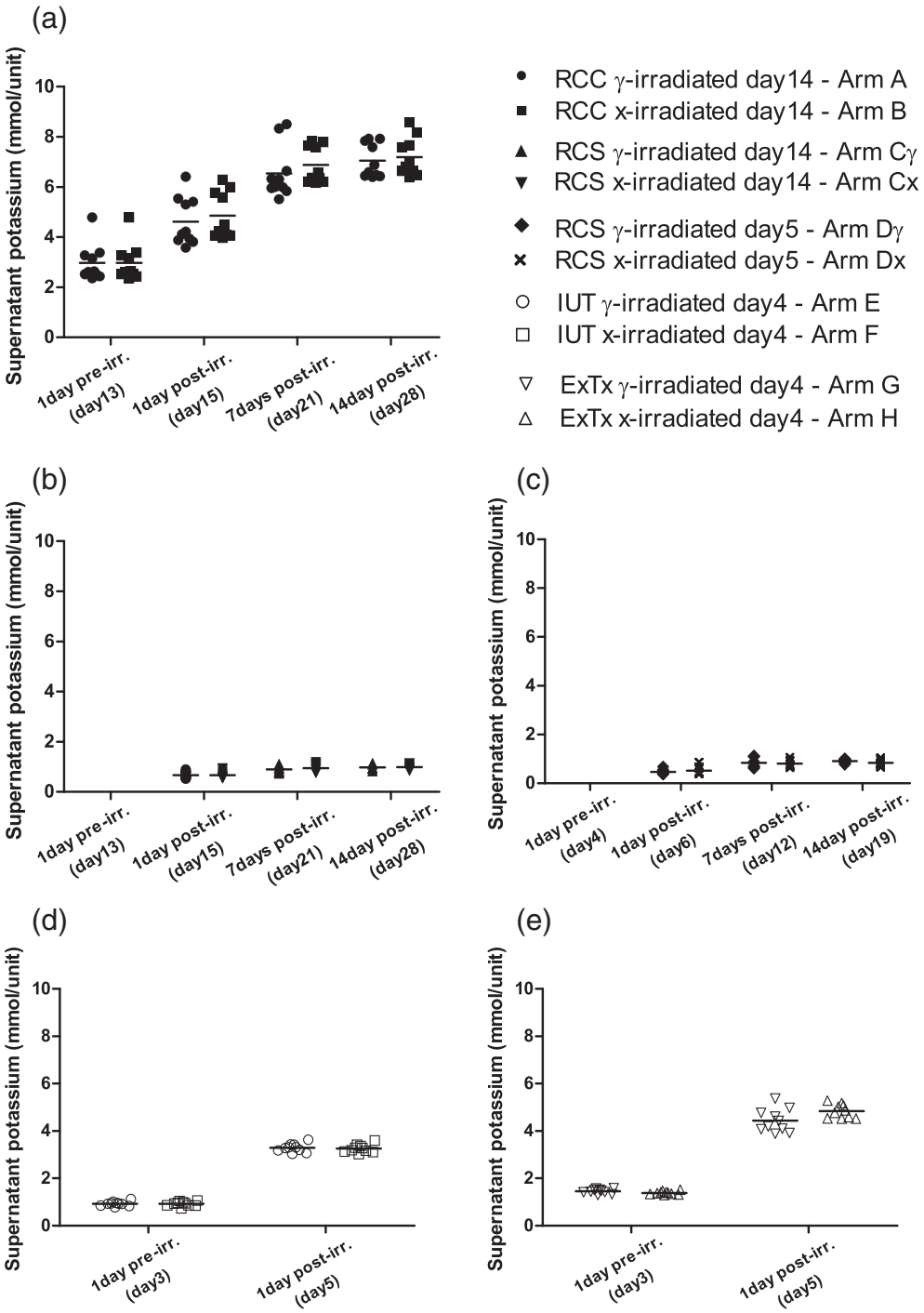


FIG. 3 Supernatant potassium concentration. Shows supernatant potassium concentration (mmol/unit) for: a. the RCC comparison arm A vs. arm B; b. the RCS irradiated late comparison arm Cγ vs. arm Cx; c. the RCS irradiated early comparison arm Dγ vs. arm Dx; d. the IUT comparison arm E vs. arm F and e. the ExTx comparison arm G vs. arm H. Solid line shows mean value at each time point and within each arm ($n = 10$). Statistical analysis deemed γ and x irradiation equivalent for all component comparisons. RCC, Red cell concentrate; RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

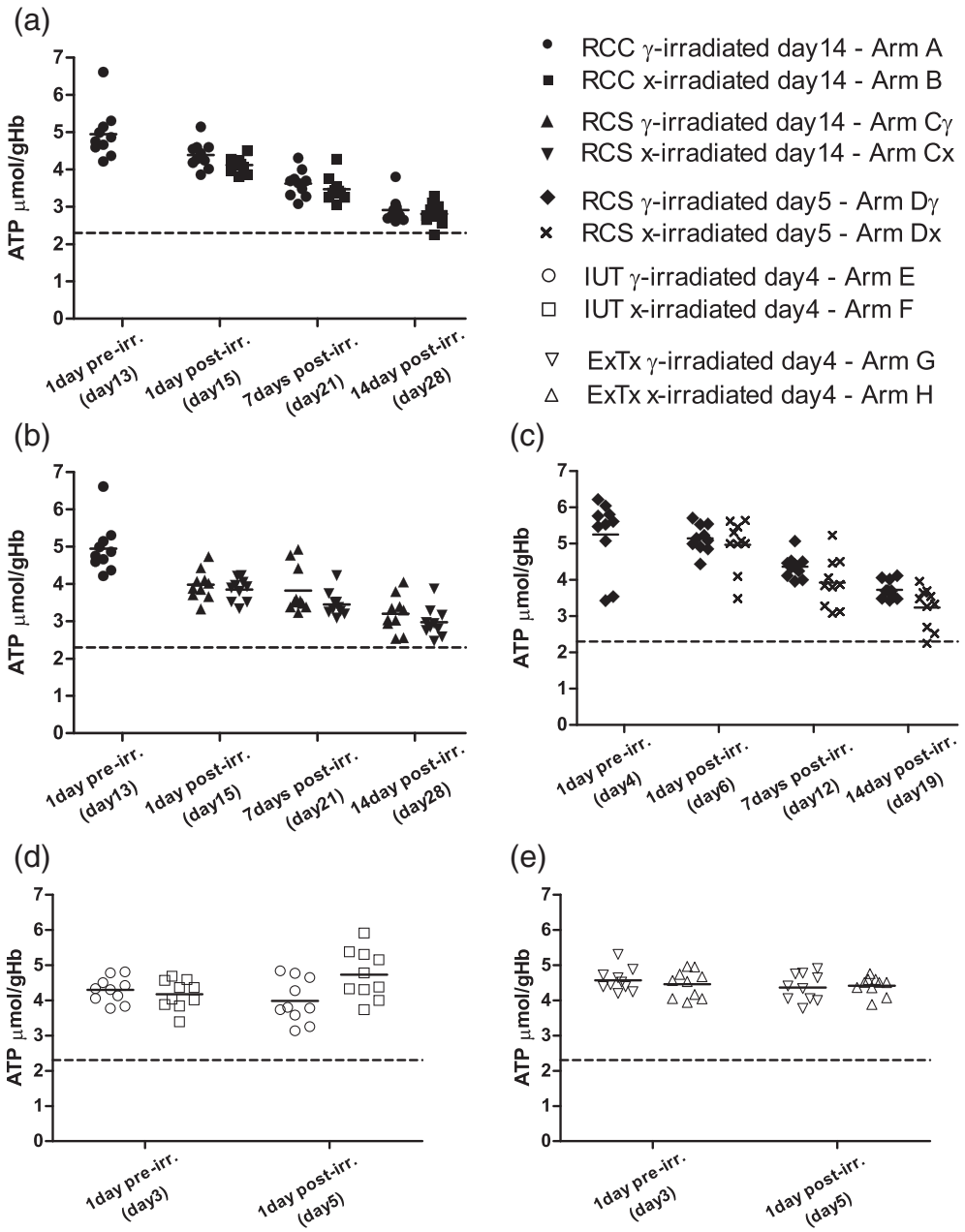


FIG. 4 Intracellular adenosine triphosphate (ATP). Shows ATP concentration (µmol/gHb) for: a. the RCC comparison arm A vs. arm B; b. the RCS irradiated late comparison arm Cγ vs. arm Cx; c. the RCS irradiated early comparison arm Dγ vs. arm Dx; d. the IUT comparison arm E vs. arm F and e. the ExTx comparison arm G vs. arm H. Solid line shows mean value at each time point and within each arm ($n = 10$). Statistical analysis deemed γ and x irradiation equivalent for RCC, RCS and ExTx comparisons but not the IUT comparison where x irradiation gave higher ATP levels than γ irradiation. Dotted line represents recommended minimum for acceptable post-transfusion survival [19]. RCC, Red cell concentrate; RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

measured with a commercially available kit (Roche Diagnostics, Welwyn Garden City, UK), both using a Synergy HT microplate reader (BioTek, Winooski, USA).

Statistical analysis

A paired *t*-test was used to compare specific parameters between two arms of the study on the first day of testing of each arm (volume and Hb), and an unpaired *t*-test was used to compare a dataset on a specific day with historic data from our laboratory, comprised of RCC in SAGM manufactured with standard procedures, using Prism 5 (GraphPad Software; San Diego, USA).

For testing whether γ and X-ray are equivalent for specific parameters between two irradiation arms of the same component, a two one-sided test (TOST) was used [16] at the 5% significance level. The underpinning model was a univariate analysis of variance (ANOVA) fitted independently to each quality storage marker across two

factors: irradiation type and days post-irradiation. A random effect was included in the model to allow for correlation between repeated measurements from each pair of pooled and split donor units. Mean values were estimated using least-squares methods. The margin of equivalence, δ, defines a range of values for which the arms are close enough to be considered equivalent [17], and it was decided that if the 90% confidence level (CL) of the mean difference between the γ and x irradiation was within 20% of the γ irradiation mean (current practice), the irradiation types will be deemed equivalent. All equivalence analyses were undertaken using SAS Enterprise Guide version 7.13 for Windows (2016 by SAS Institute Inc., Cary, NC, USA).

RESULTS

Component specification

The volume (ml), Hb (g/unit) and Hct (%) for all components were compliant to NHSBT specification. When compared, all paired arms, that is A vs B, Cγ vs Cx, Dγ vs Dx, E vs F and G vs. H, were not significantly different for volume or Hb. These parameters were taken from the first day of testing for each arm (as shown in Fig. 1); thus, arm A on day 13 was compared to arm B on day 15 as Hb and Hct are not thought to change significantly over 2 days of storage. For IUT and ExTx, the pool was tested to represent arms E and F or arms G and H, respectively. All units were within specification except one ExTx pool (Hct 55·6%). This was accepted for the purposes of this study as it was only marginally outside specification.

Quality during storage

Red cell haemolysis (Fig. 2) increased during storage in all the arms as expected. The two irradiation types were found to be equivalent for haemolysis, for all comparisons, except for the ExTx comparison (Fig. 2e) where x-irradiated red cells showed 1·6 times higher mean haemolysis than γ-irradiated red cells one-day post-irradiation. All arms complied with current UK specification for haemolysis (>75% of units must have <0·8% haemolysis) at the end of storage. Specifically, arm A, B, Cγ and Cx had 90% <0·8% haemolysis, with only one unit >0·8% haemolysis. This was the same replicate unit in all arms, that is from the same pool. Arms D – H had 100% <0·8% haemolysis, which is expected given that these units were irradiated earlier in storage (day five for arm D and day four for arms E – H).

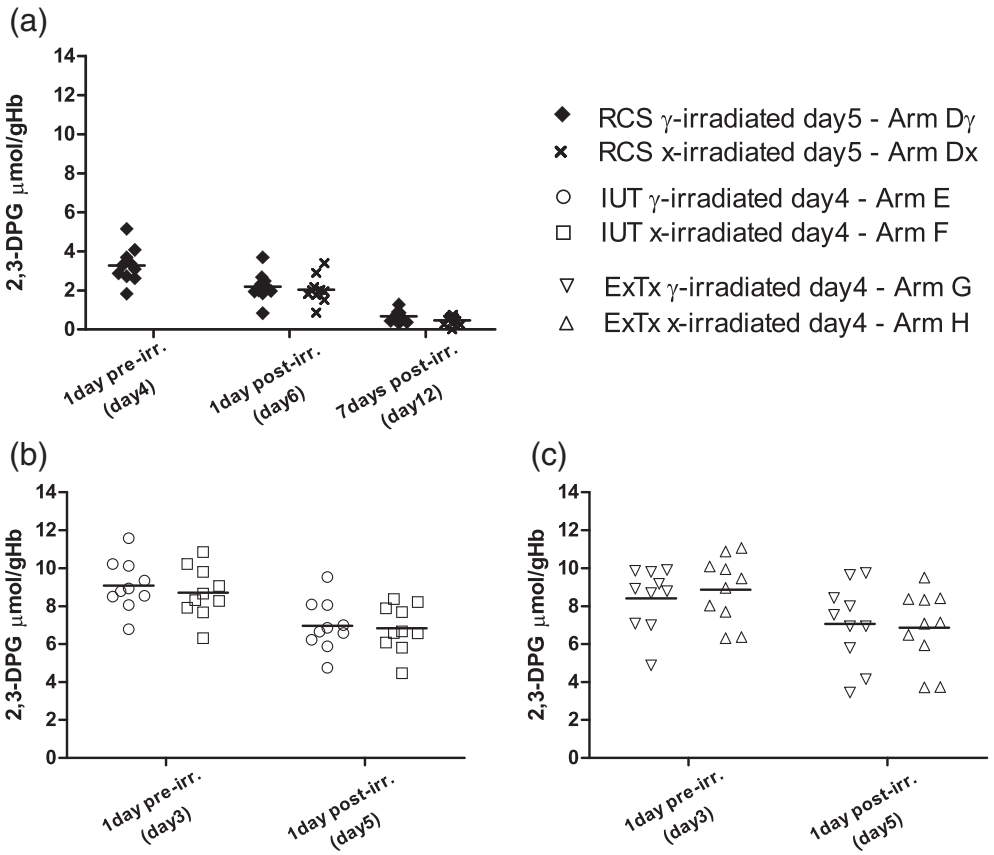


FIG. 5 Intracellular 2,3-Diphosphoglycerate (2,3-DPG). Shows 2,3-DPG concentration (µmol/gHb) for: a. the RCS irradiated early comparison arm Dγ vs. arm Dx; b. the IUT comparison arm E vs. arm F and c. the ExTx comparison arm G vs. arm H. Solid line shows mean value at each time point and within each arm ($n = 10$). Statistical analysis deemed γ and x irradiation equivalent for IUT and ExTx comparisons but not the RCS comparison. RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

Supernatant potassium released from red cells increased per unit post-irradiation as expected (Fig. 3). This parameter, as well as supernatant potassium mmol/l (not shown), was found to be equivalent between γ and x irradiation for all components studied. The pre-irradiation potassium levels are not shown for the RCS as they were not measured in the RCS units.

Mean haemolysis (0·47 [0·25–1·01]%) and potassium (68·37 [59·40–73·40] mmol/l) levels at the end of storage (day 28) in RCC arm A were within historic reference data from our laboratory for RCC units γ irradiated on day 14 and tested on day 28. Likewise, haemolysis (0·55 [0·35–0·84]%) and potassium (65·78 [56·70–69·80] mmol/l) levels in RCS arm Cγ on day 28 were not statistically different to RCS γ irradiated on day 14 and tested on day 28 from historic data. Part of the historic datasets for RCC and RCS have been previously published [18].

Adenosine triphosphate showed a decrease over storage for all arms of the study (Fig. 4). All comparisons were deemed equivalent between γ and x irradiation for this parameter except for the IUT

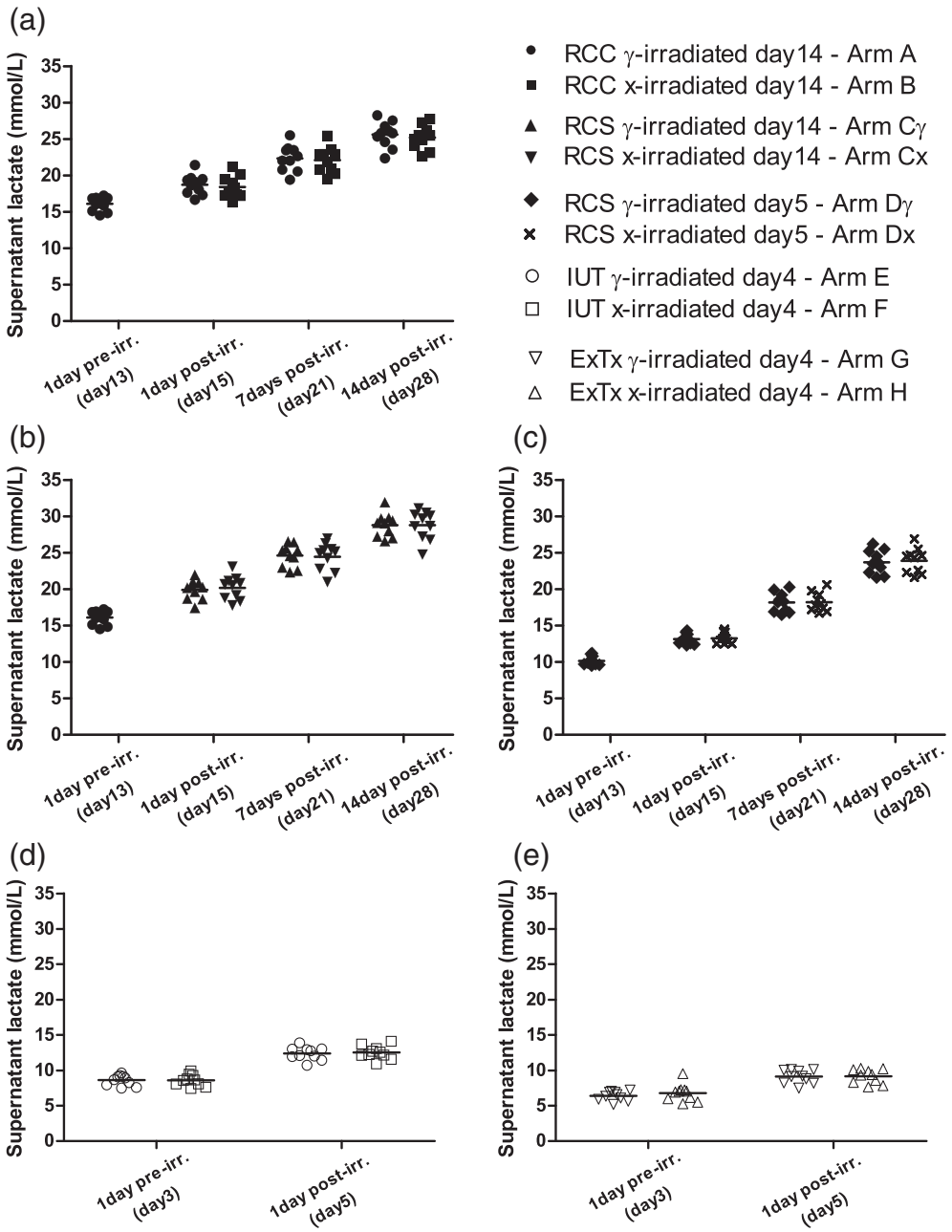


FIG. 6 Supernatant lactate. Shows supernatant lactate concentration (mmol/l) for: a. the RCC comparison arm A vs. arm B; b. the RCS irradiated late comparison arm Cγ vs. arm Cx; c. the RCS irradiated early comparison arm Dγ vs. arm Dx; e. the IUT comparison arm E vs. arm F and e. the ExTx comparison arm G vs. arm H. Solid line shows mean value at each time point and within each arm ($n = 10$). Statistical analysis deemed γ and x irradiation equivalent for all component comparisons. RCC, Red cell concentrate; RCS, Neonatal Red cell split; IUT, intra-uterine transfusion; ExTx, Neonatal Exchange Transfusion

(Fig. 4d) where equivalence was not established due to ATP levels being higher with x irradiation compared to γ irradiation. In all arms, 100% of units had ≥2·3 μmol/gHb ATP, which is the recommended minimum for acceptable post-transfusion survival [19].

Metabolite 2,3-DPG was only measured in early storage as it is depleted by day 14; therefore, only arms D – H are shown (Fig. 5). The two irradiation methodologies were deemed equivalent for the IUT and ExTx comparison, but not for the RCS comparison on day 12 of storage. However, by this time point levels were very low with means 0·67 and 0·46 μmol/gHb 2,3-DPG for γ- and x-irradiated RCS, respectively.

In this study, the two irradiation processes were found to be equivalent in terms of lactate production for all components studied (Fig. 6).

DISCUSSION

In this study, we have compared quality parameters for red cell components RCC, RCS, IUT and ExTx following γ and x irradiation over storage until the end of shelf life. RCS were irradiated both early (day five) and late (day 14) in storage, while RCC (day 14), IUT and ExTx (day 4) were irradiated only as worst case. All components in the study were acceptable for volume, Hb and Hct ranges and paired data were not statistically different for volume or Hb.

The majority of red cell quality parameters studied showed equivalence between γ and x irradiation at the 5% significance level, given the choice of equivalence margin (20% around the overall γ irradiation mean). The parameters which showed statistical non-equivalence due to worsening quality with x irradiation were haemolysis for ExTx, and 2,3-DPG for RCS with early irradiation. For haemolysis, the levels for ExTx post-irradiation were very low for both γ and X-ray, with the highest haemolysis value 24-hour post-x irradiation being 0·19%; thus, this difference is not considered clinically significant. In addition, all arms in the study complied with UK specification for haemolysis (>75% compliance at <0·8%). Overall, across all arms x irradiation haemolysis levels were on average 1–13% higher than γ irradiation, except for the ExTx arm which showed statistical non-equivalence for haemolysis. Similarly, in previous γ vs X-ray comparisons published by our group [12], there was no statistically significant difference in haemolysis levels in RCC in SAGM when γ- or x-irradiated on day 14 of storage; however, x irradiation showed overall a 16% increase in haemolysis compared with γ irradiation. This suggests a small worsening of red cell quality with x irradiation. However, the dosimetry of the x irradiator measured slightly higher central dose than the γ irradiator (35·6 Gy vs. 33·2 Gy, respectively), which may account for at least part of the higher haemolysis observed. Previous studies have shown that a central dose of 15 Gy [20] or 25 Gy [10] X-ray gave better quality red cells than a central dose of 35 Gy while maintaining a sufficient reduction of lymphocyte proliferation, suggesting red cell quality is irradiation dose-dependent. It is important to note that as we followed the more stricter UK guidelines in this study, different timings of irradiation and post-irradiation storage of products may result in higher haemolysis than shown here.

In terms of the 2,3-DPG concentration non-equivalence seen in RCS, the biggest difference between γ and x irradiation was on day 12 of storage, by which time 2,3-DPG levels were almost undetectable and reached the limits of the assay; hence, readings become more variable. Taking into account that on day 6 of storage (one-day post-irradiation), mean 2,3-DPG levels were only 7% lower with x irradiation compared with γ irradiation; this reduction can be considered clinically insignificant. Both IUT and ExTx units had higher 2,3-DPG levels than RCS, pre- and post-irradiation. This is due to the shorter time taken for WB for IUT and ExTx units to be placed at $4 \pm 2^{\circ}C$ from venepuncture (within 12 h) compared to RCC and RCS (within 27 h), as the sooner red cells start cooling, the better 2,3-DPG is preserved over storage [14].

On day six, RCS stored in SAGM had higher ATP levels than day five red cells stored in plasma (IUT and ExTx), suggesting red cells maintain ATP levels better in the presence of adenine in SAGM, a source of ATP production [21].

It is known that irradiation compromises the red cell membrane integrity directly, accelerating potassium leakage from the cells [22]. In this study, we did not see a difference in supernatant potassium levels (mmol/l or mmol/unit) between γ- or x-irradiated components beyond that defined by the margin of equivalence. This is in contrast with the study by Bashir et al. [12] which, according to the methodologies and processes at the time, found a significant increase in potassium release in x-irradiated ExTx compared with γ-irradiated units 24-h post-irradiation ($p < 0{\cdot}001$). The day of irradiation was day four for both studies. The x irradiator used in the previous study differed from the second generation equipment used here (based on its patented Quastar tube design and integrated cooling system), potentially suggesting overall improvement of the irradiation process over time.

The study design here did not include a non-irradiated arm, and for the study of RCC and RCS, we relied on the γ- irradiated arm to represent the current process within NHSBT, with results within end of storage haemolysis and supernatant potassium of historic CDL reference data. In the IUT and ExTx study, a non-irradiated arm was not needed as these components are 100% irradiated prior to issue.

In summary, despite small differences identified that suggest slight worsening of red cell quality post-x irradiation compared with γ irradiation, specifically haemolysis and 2,3-DPG levels, these differences are not considered clinically significant; therefore, x-irradiated red cell components studied here (RCC, RCS, IUT and ExTx) are of acceptable quality by the end of storage. In addition, considering the logistical benefits of x irradiators over γ irradiators, they may represent a better method for blood services.

ACKNOWLEDGEMENTS

The authors wish to thank all staff in the Component Development Laboratory who contributed to testing of the units in this work, especially Luke George, as well as all the staff in the Southampton hospital

services department who contributed to the x irradiation of the units in this work, especially Samina Mohammad and Roland Kershaw.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ORCID

Athinoula Meli https://orcid.org/0000-0003-1854-747X
Rebecca Cardigan https://orcid.org/0000-0001-6823-8937

REFERENCES

1. Foukaneli T, Kerr P, Bolton-Maggs PHB, et al. Guidelines on the use of irradiated blood components. Br J Haematol. 2020;191:704–24.
2. Bahar B, Tormey CA. Prevention of transfusion-associated graft-versus-host disease with blood product irradiation: the past, present, and future. Arch Pathol Lab Med. 2018;142:662–7.
3. UK Blood Services. Guidelines for the Blood Transfusion Services in the United Kingdom. 8th ed. London, TSO; 2013.
4. European Committee (Partial Agreement) on Blood Transfusion. Guide to the preparation, use and quality assurance of blood components. Vol. 20th. France, Council of Europe. 2020.
5. Standards for blood banks and transfusion services. AMER ASSN BLOOD BANKS; 32nd ed. Meryland; 2020.
6. Europarat, Department for the Quality of Medicines. Guide to the preparation, use and quality assurance of blood components: recommendation no. R(95) 15. 2017.
7. Australian and New Zealand Society of Blood Transfusion Ltd. Guidelines for prevention of transfusion-associated graft-versus-host disease (TA-GVHD). 1st ed. Sydney NSW 2000, Australia, Australian & New Zealand Society of Blood Transfusion Ltd.; 2011.
8. Pelszynski MM, Moroff G, Luban NL, Taylor BJ, Quinones RR. Effect of gamma irradiation of red blood cell units on T-cell inactivation as assessed by limiting dilution analysis: implications for preventing transfusion-associated graft-versus-host disease. Blood. 1994;83:1683–9.
9. Moroff G, Luban NL. The irradiation of blood and blood components to prevent graft-versus-host disease: technical issues and guidelines. Transfus Med Rev. 1997;11:15–26.
10. Janatpour K, Denning L, Nelson K, Betlach B, Mackenzie M, Holland P. Comparison of X-ray vs. gamma irradiation of CPDA-1 red cells. Vox Sang. 2005;89:215–9.
11. Task Group on Radiation Quality Effects in Radiological Protection, Committee 1 on Radiation Effects, International Commission on Radiological Protection. Relative biological effectiveness (RBE), quality factor (Q), and radiation weighting factor (w(R)). A report of the International Commission on Radiological Protection. Ann ICRP. 2003;33:1–117.
12. Bashir S, Naik F, Cardigan R, Thomas S. Effect of X-irradiation on the quality of red cell concentrates. Vox Sang. 2011;101:200–7.
13. Cookson P, Sutherland J, Cardigan R. A simple spectrophotometric method for the quantification of residual haemoglobin in platelet concentrates. Vox Sang. 2004;87:264–71.
14. Wilsher C, Garwood M, Sutherland J, Turner C, Cardigan R. The effect of storing whole blood at 22 degrees C for up to 24 hours with and without rapid cooling on the quality of red cell concentrates and fresh-frozen plasma. Transfusion. 2008;48:2338–47.
15. Lagerberg JWM, Truijens-de Lange R, de Korte D, Verhoeven AJ. Altered processing of thawed red cells to improve the in vitro quality during postthaw storage at 4 degrees C. Transfusion. 2007;47:2242–9.
16. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm. 1987;15:657–80.
17. Walker E, Nowacki AS. Understanding equivalence and noninferiority testing. J Gen Intern Med. 2011;26:192–6.
18. Meli A, New H, McAndrew M, Wiltshire M, Cardigan R. Laboratory assessment of the quality of adult and neonatal red cell concentrates manufactured from whole blood, exchange transfusion or intrauterine transfusion red cell units. Vox Sang. 2018;113:412–20.
19. Heaton WA. Evaluation of posttransfusion recovery and survival of transfused red cells. Transfus Med Rev. 1992;6:153–69.
20. Baroni F, Marraccini C, Merolle L, et al. Red blood cells metabolome changes upon treatment with different X-ray irradiation doses. Ann Hematol. 2018;97:1909–17.
21. Hess JR. An update on solutions for red cell storage. Vox Sang. 2006;91:13–9.
22. Moreira OC, Oliveira VH, Benedicto LB, et al. Effects of gamma-irradiation on the membrane ATPases of human erythrocytes from transfusional blood concentrates. Ann Hematol. 2008;87(1432–0584 (Electronic)):113–9.

How to cite this article: Meli A, Balanant MA, New HV, et al. A comparison of the effect of X and gamma irradiation on red cell storage quality. Vox Sang. 2021;1–10.

Received: 22 February 2021 | Revised: 6 May 2021 | Accepted: 14 May 2021

DOI: 10.1111/vox.13155

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Dual preparation of plasma and platelet concentrates in platelet additive solution from platelet concentrates in plasma using a novel filtration system

Tomoya Hayashi[1] | Akihiro Hayashi[2] | Yoshihiro Fujimura[1] | Mikako Masaki[1] | Tatsuya Kishikawa[2] | Hirokazu Sakaguchi[2] | Mitsunobu Tanaka[1] | Takafumi Kimura[1] | Yoshihiko Tani[3] | Yoshihiro Takihara[1] | Fumiya Hirayama[1]

[1]Japanese Red Cross Kinki Block Blood Centre, Ibaraki, Japan

[2]Advanced Materials Research Laboratories, Toray Industries, Inc., Otsu, Japan

[3]Central Blood Institute, Japanese Red Cross, Tokyo, Japan

Correspondence
Tomoya Hayashi, Japanese Red Cross Kinki Block Blood Centre, 7-5-17 Asagi Saito, Ibaraki, Osaka 567-0085, Japan.
Email: to-hayashi@kk.bbc.jrc.or.jp

Funding information
None

Abstract

Background and Objectives: Platelet concentrates suspended in a platelet additive solution (PAS-PC) are associated with a reduction in allergic response and are suitable for preparing pathogen-inactivated PC. We aimed to develop an efficient platform for the dual preparation of PAS-PC and platelet-poor plasma.

Materials and Methods: PAS-PC was prepared in six steps by using a hollow-fibre system based on cross-flow filtration: priming, loading PC, loading PAS, collection of filtered liquid (flow-through) and collection of platelets by washing with PAS followed by washing with air. In this study, the efficacy of platelet and plasma protein recovery and characteristics of recovered PAS-PC and flow-through plasma were analysed in detail.

Results: Recoveries of platelet in PAS-PC and plasma protein in the flow-through were 95.4% ± 3.7% and 61.6% ± 5.0%, respectively. The residual plasma protein in PAS-PC was 34.1% ± 2.8%. Although the expression level of CD62P, a platelet activation marker, in recovered platelets was approximately 1.2-fold of that in original platelets, swirling patterns were well retained, and aggregation in PAS-PC was not visible. Agonist-induced aggregabilities, platelet morphology and hypotonic shock recovery were conserved. The patterns of plasma protein and lipoprotein in the flow-through were comparable with those in the original PCs. The multimeric pattern analysis of VWF remained unaltered.

Conclusion: We propose a highly efficient preparation system that enables the simultaneous production of PAS-PC and platelet-poor plasma. It also achieves a high recovery of functionally well-retained platelets with very low activation.

KEYWORDS
allergic transfusion reactions, hollow fibre, plasma protein, platelet additive solution, platelet concentrates

INTRODUCTION

Transfusion of platelet concentrates (PCs) is indicated in patients with thrombocytopenia due to acute life-threatening bleeding, chemotherapy-related marrow dysfunction, heightened consumption and less frequently to cease bleeding in congenital platelet disorders regardless of platelet counts [1]. However, transfusion of PCs is associated with various allergic transfusion reactions (ATRs),

with a frequency of 1%–4% [2, 3]. ATRs most commonly manifest as urticaria, pruritus, erythematous rash, angioedema, bronchospasm and/or hypotension. These manifestations vary in severity but are most commonly mild, presenting only with localized pruritus and/or urticaria. The prediction of ATRs is rather difficult, and pre-medication may generally be inconceivable.

To reduce ATRs, the clinical application of platelet-additive-solution suspended PC (PAS-PC) was first launched in 1991 in Sweden, and second- to fifth-generation PAS components for improving the quality of PC during storage were developed [4–7]. Because of the reduction in the plasma component (~35% of the original plasma) in PAS-PCs, the frequency of PC-related ATRs can be significantly decreased to about half of that in non-treated PCs [8, 9].

Buffy coat–derived blood products are widely used globally [10–13]. Originally, in the European Union, a centrifugation-based system using the combination of Atreus with TACSI (stands for Terumo Automated Centrifuge and Separator Integration; Terumo Medical) systems [14] was introduced to prepare PAS-PCs, where pooled PCs and a mixture of buffy coats prepared from five to six individual whole blood parts, which were re-suspended in plasma, were used as the initial material. More recently, a fully automated protocol for centrifugation-based systems has been introduced, which is expected to increase the volume of source plasma and address the expanded global need for immunoglobulins (IgGs) in recent years. In recent decades, collecting plasma-reduced PCs by apheresis has become more popular [9, 15]. However, visible aggregates are sometimes observed even in PAS-PC like in plasma-PC, although the incidence ranges widely from almost zero to 10%–15% [15, 16]. The presence of aggregates, in both, could be a sign of bacterial contamination. Therefore, presence of visible aggregates poses an issue to countries and regions, including Japan, where pathogen inactivation techniques and/or bacterial screening strategies have not been implemented. Platelet activation by processing during centrifugation and/or shear stress (pumps) during collection are considered plausible causes of platelet aggregation [15].

Utilizing a hollow-fibre system devoid of a centrifugation step, we recently reported the successful preparation of washed PCs and HLA-eliminated PCs from plasma-suspended PCs, with very low platelet activation and high platelet count recovery [17, 18]. In line with these studies, we report here an efficient platform for dual preparation of PAS-PC and platelet-poor plasma using a hollow-fibre system coupled with a cross-flow filtration technique. In this pilot study, we aimed to evaluate PAS-PCs and flow-through plasma fraction and propose that the novel method is the third choice for PAS-PC preparation.

MATERIALS AND METHODS

Chemicals

The chemicals purchased for this study included (1) phycoerythrin (PE)-conjugated anti-human CD62P (clone: AC1.2, mouse IgG, BD Biosciences, Tokyo, Japan), (2) PE-conjugated mouse IgG (clone: MOPC-21, BD Biosciences, Tokyo, Japan), (3) peridinin chlorophyll protein complex (PerCP)-conjugated anti-human CD61 (clone: RUU-PL 7F12, mouse IgG, BD Biosciences, Tokyo, Japan), (4) horseradish peroxidase–conjugated polyclonal VWF antibody (Dako, Glostrup, Denmark), (5) collagen reagent (Horm collagen, Moriyasangyo, Tokyo, Japan) and (6) TRAP (thrombin receptor activator peptide 6, SFLLRN, Abcam, Tokyo, Japan). PAS-E was purchased from Grifols S.A. (Barcelona, Spain).

Preparation and storage of platelet concentrates

Leuco-reduced (<1 × 10^6/bag) PCs were collected from 10 volunteer donors using the standard aphaeresis procedure using Trima (TerumoBCT) or CCS (Haemonetics Corporation) aphaeresis instruments. The harvested PCs contained more than 2 × 10^{11} platelets per bag and were irradiated with 15-Gy x-rays. The PCs were stored at 22°C with agitation before use within 4 days. This study was approved by the ethics committee of the Japanese Red Cross Blood Institute (2019-005).

Preparation of PAS-PC

To prepare the PAS-PCs, 200 ml of PCs were applied to a hollow-fibre column (Toray Industries, Inc., Otsu, Japan), which was used in our previous study for the preparation of washed PCs [17]. The dimensions of this device are approximately 30 cm (length) and 9.7 cm (diameter), containing 792 hollow fibres inside. As shown in Figure 1, a PC bag, 300 ml PAS-E bag, storage bag (PAS-PC collection bag), waste bag and flow-through plasma collection bag were assembled using a 3 × 6 SR tube (AS one) to connect a hollow-fibre column, and two roller pumps (FP-300-1515, AS ONE Corporation, Osaka, Japan) were set; one was set upstream of the hollow-fibre column and another between the column and the flow-through bag. PAS-PC was prepared by six distinct steps that switch direction and flow rate manually: (1) priming, (2) loading PC, (3) loading PAS, (4) collection of flow-through, (5) collection of platelets by washing with PAS and (6) collection of platelets in the residual washing PAS present in the column by air. To achieve stable filtration, the pump flow rate was set at 100/s at the inlet of the hollow fibre, and the filtration rate was set at 70% using the second pump. A newly assembled circuit including bags, hollow-fibre and tubes was used for each run. An aliquot (~5 ml) of the original PCs and the resulting products were collected and used for the analyses described below.

Platelet counts and pH

Platelet counts and pH were determined using a cell counter (Sx-800, Sysmex, Tokyo, Japan) and blood gas analyser (Model 348, Siemens Medical, Tokyo, Japan), respectively. Platelet recovery was calculated as the ratio of the number of platelets in the PAS-PC products to that in the original PCs.

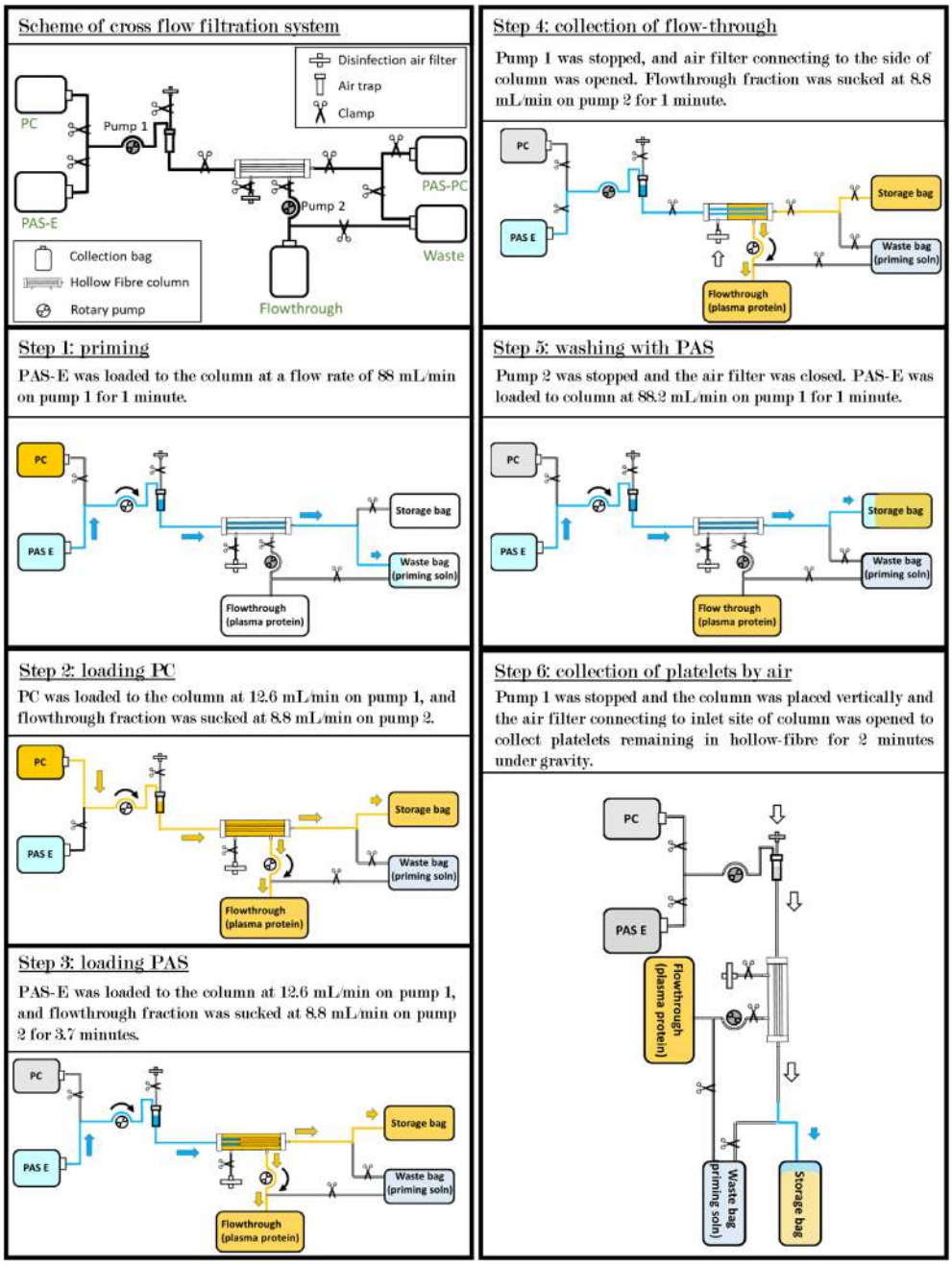


FIGURE 1 Outline of cross-flow filtration system. The cross-flow system consists of an assembled hollow fibre module, one air trap, two rotary pumps, two air filters, five collection bags and PC with silicon tubes. The procedure consists of six distinct steps using a hollow-fibre system by manually switching the flow direction and flow rate. In step 1, PAS-E is loaded onto the column at a flow rate of 88 ml/min on pump 1 for 1 min. The fluid is collected in a waste bag. In step 2, PC is loaded to the column at 12.6 ml/min on pump 1 and at 8.8 ml/min on pump 2 for 15.9 min. Fluids are collected in a PAS-PC bag and flow-through a collection bag. In step 3, PAS-E is loaded to the column at 12.6 ml/min on pump 1 and at 8.8 ml/min on pump 2 for 3.7 min. In step 4, pump 1 is stopped and the airport on the outer side of the column is opened and then flows through the outside of the hollow fibre and is collected at 8.8 ml/min on pump 2 for 1 min. In step 5, pump 2 is stopped, and the airport outside the column is closed. Then, PAS-E is loaded to the column at 88.2 ml/min on pump 1 for 1 min. In step 6, pump 1 is stopped, the column stood vertically, and the airport on the upper-side outlet is opened to collect platelets in the hollow fibre for 2 min under normal gravity [Colour figure can be viewed at wileyonlinelibrary.com]

Agonist-induced platelet aggregation

Agonist-induced platelet aggregation was measured as previously described [18]. Briefly, platelet samples were suspended in an equal mixture of type AB plasma and PAS-E to obtain a final platelet count of $300 \times 10^3/\mu l$ (PRP). Calcium chloride at final concentrations of 4 mM and agonists were added to 200 µl of the diluted platelet samples. ADP, collagen and ADP plus collagen at final concentrations of 20 µM, 5 µg/ml and 5 µM plus 2.5 µg/ml, respectively, were added to the samples. Agonist-induced platelet aggregation was assessed using a

TABLE 1 In vitro properties of original PC, PAS-PC and flow-through

	Original PC	PAS-PC	*p* values	Recovery[a]	Flow-through	*p* values	Recovery[b]
Volume (ml)	201 ± 1	203 ± 10	0.59	–	170 ± 8*	<0.001	–
PLT count ($\times 10^4$/μl)	113 ± 15	107 ± 13	0.02	–	–		–
Total platelets ($\times 10^9$)[c]	227 ± 31	217 ± 30*	0.004	95% ± 4%	–		–
pH (at 37°C)	7.22 ± 0.13	7.22 ± 0.04	1		–		–
CD62P-positive platelets (%)[d]	17.2 ± 6.9	20.4 ± 7.7*	<0.001	–	–		–
Protein concentration (g/dl)	5.8 ± 0.4	2.0 ± 0.1*	<0.001	–	4.1 ± 0.4*	<0.001	–
Total protein (g)	11.4 ± 0.7	4.0 ± 0.7*	<0.001	34.1% ± 2.8%	7.0 ± 0.8*	<0.001	61.6% ± 5.0%

Note: Values are shown as mean ± SD ($n = 10$).
*Significantly different from original PCs at $p < 0.005$. [a]Divide the total platelet number of PAS-PC by that of the original PC.
[b]Divide the total protein of flow through by that of the original PC. Total protein; multiply the protein concentration by the volume.
[c]Multiply the platelet count by the volume.
[d]The %-CD62P positive platelets were raised from 13.1% to 94.1% after TRAP stimulation at the final concentration of 10 nM.

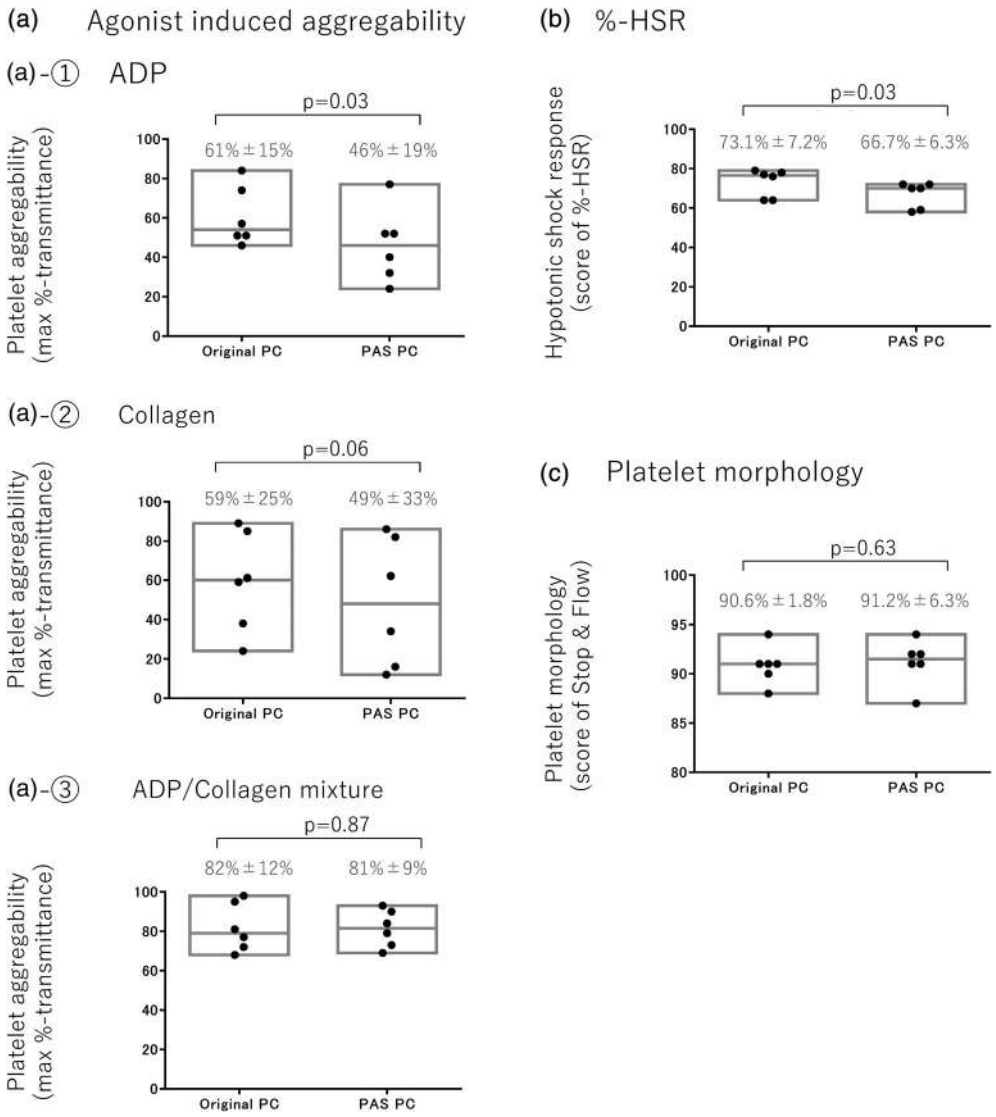


FIGURE 2 In vitro platelet quality tests. Agonist-induced platelet aggregation (a), hypotonic shock response (%-HSR) (b) and stop and flow (c). (a) PRP was stimulated with ADP, collagen and ADP plus collagen at final concentrations of 20 μM, 5 μg/ml and 5 μM plus 2.5 μg/ml, respectively. (a–c) Six independent experiments (circles) and the median, maximum and minimum (vertical line and box) are shown. Significant differences (*p*-values) were determined by Wilcoxon signed-rank test

Hematracer 912 (Tokyo Photoelectric, Tokyo, Japan) for 7 min using AB plasma and PAS-E mixture as a reference for 100% transmittance.

Hypotonic shock response and platelet morphology

The HSR was determined using the standard method described by Holme and colleagues [19]. Briefly, the samples (PRPs) were mixed with distilled water and physiological normal saline followed by measurement of %transmittance at 550 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) for 7 min using physiological normal saline as a Reference [20]. To determine platelet morphology, PRPs were subjected to stop and flow methods described previously using a modified aggregometer (Hematracer 801, NBS, Tokyo, Japan). Briefly, we recorded the %transmittance of the PRP samples under stirring (T'800) and non-stirring (T0) conditions, and E800/E0 was calculated as the morphology score.

Surface expression of CD62P

Fifty microliters of PAS-PCs and aliquots of original PCs were fixed with 1% PFA and stored at 4°C for at least 2 h. CD62P expression was measured as described previously [21]. Fixed platelets were washed with PBS, followed by labelling with both PerCP-conjugated anti-CD61 and PE-conjugated anti-human CD62P antibodies (or PE-conjugated mouse IgG as a negative control) by incubating for 20 min at room temperature in the dark. The samples were then washed with PBS and subjected to flow cytometric analysis. The percentage of CD62P-positive cells within a region gate of CD61-positive cells was then measured using a cell analyser SA3800 (Sony, Tokyo).

Plasma analyses

The original PC, PAS-PC and flow-through were centrifuged at 7500 g for 10 min. Each aliquot of the supernatants was used to determine the protein concentration using the Pierce BCA protein assay kit (Thermo Scientific, IL). The residual sample was divided into two; one was stored at 4°C for analyses of A/G ratio, protein fraction and lipoprotein fractions; while the other was stored at −20°C for VWF-multimer analysis. The former three analyses were performed by an outsourced Japanese laboratory (SRL Inc, Tokyo, Japan). VWF multimer analysis was performed according to the method described by Budde et al. [22, 23], with a minor modification by Naito et al. and Horiuchi et al. [24, 25]. The supernatants were subjected to electrophoresis in 1% agarose gel on 3 mA for 12 h at 4°C. Proteins were transferred to a polyvinylidene difluoride membrane by capillary blotting. The blots were incubated with HRP-conjugated polyclonal rabbit anti-human VWF antibody (Dako, CA). The protein bands were visualized using western lightning ECL (PerkinElmer, Boston, MA) and detected by Image Quant LAS500 (GE Healthcare, Tokyo, Japan). Relative levels of VWF fragments were quantified by densitometry using Image J software (National Institutes of Health, Bethesda, MD). Small, middle and large multimer bands were defined as five bands that were closest to the negative electrode, 6–10 bands and >10 bands, respectively, in accordance with a previous report [25].

Statistical analysis

The statistical significance of the differences between the various samples was analysed using the Wilcoxon signed-rank test, with $p < 0.005$ being considered as significant.

RESULTS

Preparation of PAS-PC and flow-through plasma with a hollow-fibre column system

The whole procedure was carried out at room temperature (ranging approximately from 20 to 24°C) for about 30 min. In all 10 experiments, there was no visible aggregate in the PAS-PC products. The in vitro properties of the original PCs, PAS-PCs and flow-throughs are shown in Table 1. The volume of PAS-PC was comparable to that of the original PC, and the platelet count decreased by approximately 5%. Therefore, the platelet recovery in PAS-PC was 95.4% ± 3.7%. No platelet was detected in the flow-through fraction (<1.0 × 10^4/μl). The values of pH were comparable. The

TABLE 2 Proportion of plasma proteins in original PC and flow-through

	Original PC	Flow-through	*p* value[a]
Plasma protein fraction			
Albumin	59.2% ± 2.8%	59.7% ± 2.8%	0.06
Alfa 1 globulins	3.3% ± 0.5%	3.2% ± 0.5%	0.78
Alfa 2 globulins	8.7% ± 1.1%	8.6% ± 1.0%	0.38
Beta 1 globulins	6.3% ± 0.6%	6.1% ± 0.7%	0.14
Beta 2 globulins	10.5% ± 0.9%	10.4% ± 1.0%	0.34
Gamma globulins	12.1% ± 1.5%	12.0% ± 1.6%	0.38
Lipoprotein fraction			
HDL	20.5% ± 5.6%	20.8% ± 6.0%	0.66
LDL	44.7% ± 6.1%	43.2% ± 5.3%	0.31
MIDBAND	14.7% ± 7.6%	13.2% ± 8.0%	0.50
VLDL	19.8% ± 2.4%	21.7% ± 3.4%	0.36
A/G ratio	1.5 ± 0.2	1.5 ± 0.2	0.25

Note: Values are shown as mean ± SD ($n = 10$). HDL, LDL, MIDBAND, VLDL and A/G ratio mean high-density lipoprotein, low-density lipoprotein, mid-band lipoprotein, very low-density lipoprotein and albumin–globulin ratio, respectively.

[a]*p*-values were determined using Wilcoxon signed-rank test.

percentage of CD62P-positive platelets of the irradiated platelets in PAS-PC was slightly but significantly increased, indicating some additional activation of platelets during the process.

Characterization of platelets in PAS-PC

No visible platelet aggregates were observed in PAS-PC. The results of agonist-induced platelet aggregability, HSR and platelet morphology are summarized in Figure 2(a–c).

As shown in Figure 2(a), after PAS-PC preparation, the maximum platelet aggregation elicited by ADP stimulation slightly decreased, but that by collagen was not substantially reduced. The lower sensitivity to ADP in PAS compared to that in plasma may be an effect of PAS as it contains lower levels of apyrase [26]. Therefore, the aggregation induced by ADP plus collagen was not reduced substantially, indicating that platelet aggregability was well retained. Furthermore, the percentage of HSRs slightly decreased after PAS-PC preparation, but there was no significant difference in platelet morphology, which was measured as a score of stop and flow (Figure 2(b, c)).

Characterization of plasma proteins in the flow-through fraction

The proportions of plasma proteins, including albumin, a1−/a2-globulin, b1−/b2-/r-globulins and lipoprotein fractions including HDL, LDL, MID-BAND and VLDL were almost indistinguishable between the original PCs and flow-through fractions (Table 2). We then investigated the molecular structure of plasma proteins using VWF as a representative using multimeric analysis. Densitometric analysis was used to determine the ratio of large, medium and small VWF multimers. Figure 3(b) shows no significant changes between the original PCs and flow-through fractions. When fractionated, the protein concentration in the flow-through gradually increased in the first 63 ml up to that found in the original PC and remained high for the next ~105 ml (Figure S1); the recovery rate of the high protein concentration fraction was approximately 53%.

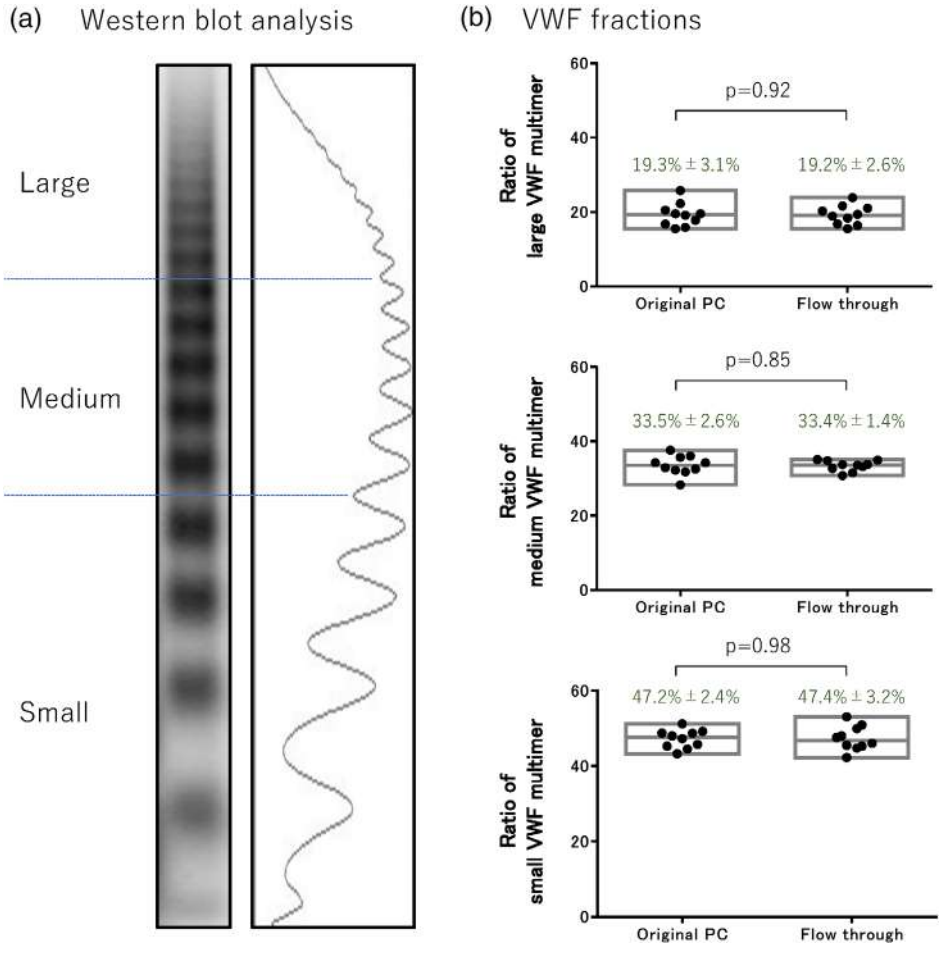


FIGURE 3 VWF multimer analysis. (a) Representative western blotting and VWF fraction analysis. (b) Three VWF fractions, large, medium and small, were quantified, and the ratio of fractions is shown. Eleven independent experiments (circles) and the median, maximum and minimum (vertical line and box) are shown. The values of the mean ± 1 SD are also shown. Significant differences (*p*-values) were determined by Wilcoxon signed-rank test [Colour figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

A hollow-fibre system has recently been developed in Japan for the preparation of washed PCs and HLA-eliminated PCs from PCs suspended in plasma [17, 18]. This development drove us to explore the possibility that the same hollow-fibre system in combination with the cross-flow technique can achieve simultaneous dual preparations of PAS-PC and flow-through plasma. It was demonstrated that platelets were efficiently recovered, barely activated and functionally well preserved, while approximately 34% and 62% of plasma protein were recovered in PAS-PC and flow-through, respectively. Our PAS-PCs and high concentration fraction of the flow-through fulfil the present regulations of Japan, as platelet product and source plasma, respectively.

PAS-PCs collected as plasma-reduced PCs by apheresis and the use of PAS as a storage medium in platelet production are occasionally associated with a higher incidence of small platelet aggregates [15]. Interestingly, in this study, which used a hollow-fibre system, no aggregates were observed. There are three possibilities in this respect. First, we simply used PCs that did not contain visible aggregations as the original PCs. Second, preparations using hollow fibre resulted in a much lower physiological stress than that using centrifugation, in agreement with Flesch et al. [27], who stated that centrifugal forces and shear stress during the centrifugation process might cause platelet activation and aggregation. Third, as some reports mentioned, donor-related parameters may be one of the causes of platelet aggregation [8, 28]. Therefore, a larger study is necessary to clarify the issue of aggregation in the future.

One of the distinct advantages of PAS-PCs is that UV irradiation for pathogen reduction can be more efficiently adopted to PAS-PCs than plasma PCs because of their higher UV light transparency. However, pathogen reduction techniques usually elicit substantial platelet activation [29, 30]. In this regard, it is conceivable that if platelets are less activated in the process of PAS-PC preparation, they would be less activated in the subsequent pathogen reduction process as well, and hence, our system would provide good platelet sources with reduced pathogens.

The pore size of the hollow fibres used in this study was 1000 nm; thus, one may be concerned that plasma proteins might adsorb onto the hollow fibres and/or might destroy the molecular structure of macromolecular plasma proteins. The recovery of plasma protein in the flow-through fraction was high at 61.6%, whereas that in PAS-PC was 34.1%. This result indicates that the hollow-fibre module absorbs plasma protein minimally. The proportion of several representative proteins, fractions of plasma proteins, IgG and lipoproteins did not significantly differ between the original PCs and flow-through fractions. In addition, although we examined only VWF multimers as a representative of macromolecular plasma proteins with MW ranging from 500 to 15,000 kD in normal plasma, the multimer ratio of VWF followed by densitometry was well retained, and the ratios of large, medium and small multimers of VWF were the same between plasmas of original PCs and flow-through plasmas. These results indicate that the pores, which opened within the hollow fibres, did not interfere with the passage of plasma proteins, including macromolecular proteins.

Plasma derivatives, especially r-globulins, are widely applicable for a variety of therapies and are used for cases with immune deficiencies such as immune thrombocytopenia, Kawasaki disease, Guillain–Barre syndrome, chronic inflammatory demyelinating polyneuropathy, lupus, myositis and neurological diseases such as myasthenia gravis or multiple sclerosis [31, 32]. Patients who received bone marrow transplants may also be administered with IVIg to prevent infections and other rare diseases. One of the limitations of this study was that the recovery of flow-through fractions with a high concentration of plasma proteins as the original plasma was only approximately 53% (105 ml from 200 ml in PCs).

Another limitation of this study is that all experiments were performed under open-air conditions. As a result, we were unable to examine the in vitro characteristics of PAS-PCs during storage. However, we assumed that there would be no substantial difference in platelet function between PAS-PCs and original PCs for the following reasons. First, among several characteristics, the percentage of CD62p-positive platelet PAS-PCs was higher than that of the original PCs, but the difference was minimal (1.2-fold). Although the HSR was decreased (0.9-fold), the p-value was only 0.03. There was no significant difference in the pH between original PCs and PAS-PCs. Because PAS-PCs contain PAS-derived buffer solution with phosphate and/or bicarbonate, the pH of PAS-PC may be more stable than that of original PCs. Moreover, the ratio of plasma to PAS in PAS-PC was approximately 2–1 (66%/34%). Second, it was reported that even though platelets were slightly activated during the preparation process, storage in PAS could mitigate such transient platelet activation [6, 8]. In a separate study, we plan to develop an automated and closed system with sterile equipment, including modules, junction connectors, bags, hollow fibre columns and tubes, which would allow us to examine the in vitro characteristics of PAS-PCs during storage.

Taken together, this study indicates that our cross-flow technique is a useful and efficient platform for the dual preparation of PAS-PC and platelet-poor plasma. Therefore, we assume that the hollow-fibre system can be used for segregating blood components in the future.

ACKNOWLEDGEMENTS

We would like to thank Dr. Tanaka for supporting this study, Dr. Fuchizaki for the advice regarding experimental procedures, and all the team members in the Kinki Block Blood Centre, especially in the donation section, who helped prepare the various blood samples used in this study. The authors thank Editage (www.editage.jp) for the English language review.

T.H., T.K. and F.H. conceived the project; T.H. and M.M. carried out the experiments; T.H., A.H., Y.F., T.K. and H.S. designed and performed the experiments; T.H., T.K., M.T., T.K., Y.T., Y.T. and F.H. analysed the data, drafted and critically revised the manuscript. All authors discussed the results and commented on the manuscript. All authors have approved the publication of the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Tomoya Hayashi https://orcid.org/0000-0002-1776-0052
Fumiya Hirayama https://orcid.org/0000-0001-6518-2085

REFERENCES

1. Kumar A, Mhaskar R, Grossman BJ, Kaufman RM, Tobian AAR, Kleinman S, et al. Platelet transfusion: a systematic review of the clinical evidence. Transfusion. 2015;55:1116–27. https://doi.org/10.1111/trf.12943.
2. Enright H, Davis K, Gernsheimer T, McCullough JJ, Woodson R, Slichter SJ. Factors influencing moderate to severe reactions to PLT transfusions: experience of the TRAP multicentre clinical trial. Transfusion. 2003;43:1545–52. https://doi.org/10.1046/j.1537-2995.2003.00529.x.
3. Hirayama F. Current understanding of allergic transfusion reactions: incidence, pathogenesis, laboratory tests, prevention and treatment. Br J Haematol. 2013;160:434–44. https://doi.org/10.1111/bjh.12150.
4. Gulliksson H. Platelet storage media. Vox Sang. 2014;107:205–12. https://doi.org/10.1111/vox.12172.
5. Ashford P, Gulliksson H, Georgsen J, Distler P. Standard terminology for platelet additive solutions. Vox Sang. 2010;98:577–8. https://doi.org/10.1111/j.1423-0410.2009.01298.x.
6. Tynngård N, Trinks M, Berlin G. In vitro quality of platelets during prolonged storage after washing with three platelet additive solutions. Vox Sang. 2012;102:32–9. https://doi.org/10.1111/j.1423-0410.2011.01500.x.
7. Bashir S, Kemsley K, Min K, Swann ID, Cardigan R. Platelet storage in more than 90% additive solution containing glucose and bicarbonate has the potential to increase shelf life. Transfusion. 2018;58:2959–68. https://doi.org/10.1111/trf.14962.
8. Heaton WA. Editorial. Costs and benefits of PAS platelets: a mix of science, quality, and value. Transfusion. 2013;53:2597–602.
9. Tobian AA, Fuller AK, Uglik K, Tisch DJ, Borge PD, Benjamin RJ, et al. The impact of platelet additive solution apheresis platelets on allergic transfusion reactions and corrected count increment (CME). Transfusion. 2014;54:1523–9. https://doi.org/10.1111/trf.12498.
10. Janetzko K, Klüter H, Waeg GV, Eichler H. Fully automated processing of buffy-coat–derived pooled platelet concentrates. Transfusion. 2004;44:1052–8. https://doi.org/10.1111/j.1537-2995.2004.03296.x.
11. Larsson S, Sandgren P, Sjödin A, Vesterinen M, Gulliksson H. Automated preparation of platelet concentrates from pooled buffy coats: in vitro studies and experiences with the OrbiSac system. Transfusion. 2005;45:743–51. https://doi.org/10.1111/j.1537-2995.2005.04096.x.
12. Vetlesen A, Mirlashari MR, Ezligini F, Kjeldsen-Kragh J. Evaluation of platelet activation and cytokine release during storage of platelet concentrates processed from buffy coats either manually or by the automated OrbiSac system. Transfusion. 2007;47:126–32. https://doi.org/10.1111/j.1537-2995.2007.01075.x.
13. Gammon RR, Devine D, Katz LM, Quinley E, Wu YY, Rowe K, et al. Buffy coat platelets coming to America: are we ready? Transfusion. 2020;61:1–7.
14. Cid J, Magnano L, Lozano M. Automation of blood component preparation from whole blood collections. Vox Sang. 2014;107:10–8. https://doi.org/10.1111/vox.12131.
15. Ringwald J, Antoon M, Eckstein R, Cardoso M. Residual aggregates in platelet products: what do we know? Vox Sang. 2014;106:209–18. https://doi.org/10.1111/vox.12089.
16. van der Meer PF, Dumont LJ, Lozano M, Bondar N, Wong J, Ismay S, et al. Aggregates in platelet concentrates. Vox Sang. 2015;108:96–100. https://doi.org/10.1111/vox.12184.
17. Tanaka S, Hayashi T, Sugaya S, Osabe M, Ueno Y, Tani Y, et al. A hollow-fibre column system to effectively prepare washed platelets. Vox Sang. 2015;109:239–47. https://doi.org/10.1111/vox.12284.
18. Hayashi T, Aminaka R, Fujimura Y, Koh Y, Sugaya S, Hayashi A, et al. A more efficient preparation system for HLA-eliminated platelets. Vox Sang. 2020;115:159–66. https://doi.org/10.1111/vox.12859.
19. Holme S, Heaton WA, Courtright M. Improved in vivo and in vitro viability of platelet concentrates stored for seven days in a platelet additive solution. Br J Haematol. 1987;66:233–8. https://doi.org/10.1111/j.1365-2141.1987.tb01304.x.
20. Summerer MH, Genco PV, Katz AJ. The response of human platelets to hypotonic stress: direct measurement of volume change. Ann Clin Lab Sci. 1978;8:447–52.
21. Hayashi T, Tanaka S, Hori Y, Hirayama F, Sato EF, Inoue M. Role of mitochondria in the maintenance of platelet function during in vitro storage. Transfus Med. 2011;21:166–74. https://doi.org/10.1111/j.1365-3148.2010.01065.x.
22. Budde U, Schneppenheim R, Plendl H, Dent J, Ruggeri ZM, Zimmerman TS. Luminographic detection of von Willebrand factor multimers in agarose gels and on nitrocellulose membranes. Thromb Haemost. 1990;63:312–5.
23. Budde U, Pieconka A, Will K, Schneppenheim R. Laboratory testing for von Willebrand disease: contribution of multimer analysis to diagnosis and classification. Semin Thromb Hemost. 2006;32:514–21. https://doi.org/10.1055/s-2006-947866.
24. Naito N, Mizuno T, Nishimura T, Kishimoto S, Takewa Y, Eura Y, et al. Influence of a rotational speed modulation system used with an implantable continuous-flow left ventricular assist device on von Willebrand factor dynamics. Artif Organs. 2016;40:877–83. https://doi.org/10.1111/aor.12666.
25. Horiuchi H, Doman T, Kokame K, Saiki Y, Matsumoto M. Acquired von Willebrand syndrome associated with cardiovascular diseases. J Atheroscler Thromb. 2019;26:303–14.
26. Keuren JF, Cauwenberghs S, Heeremans J, de Kort W, Heemskerk JWM, Curvers J. Platelet ADP response deteriorates in synthetic storage media. Transfusion. 2006;46:204–12. https://doi.org/10.1111/j.1537-2995.2006.00702.x.
27. Flesch BK, Adamzik I, Steppat D, Miller J, Carstensen L, Schapke M, et al. Paired crossover study of two plateletpheresis systems concerning platelet product quality and donor comfort. Transfusion. 2010;50:894–901. https://doi.org/10.1111/j.1537-2995.2009.02502.x.
28. Jiménez TM, Patel SB, Pineda AA, Tefferi A, Owen WG. Factors that influence platelet recovery after transfusion: resolving donor quality from ABO compatibility. Transfusion. 2003;43:328–34.
29. Kaiser-Guignard J, Canellini G, Lion N, Abonnenc M, Osselaer J, Tissot J. The clinical and biological impact of new pathogen inactivation technologies on platelet concentrates. Blood Rev. 2014;28:235–41. https://doi.org/10.1016/j.blre.2014.07.005.
30. Hechler B, Ohlmann P, Chafey P, Ravanat C, Eckly A, Maurer E, et al. Preserved functional and biochemical characteristics of platelet components prepared with amotosalen and ultraviolet a for pathogen inactivation. Transfusion. 2013;53:1187–200. https://doi.org/10.1111/j.1537-2995.2012.03923.x.
31. Perez EE, Orange JS, Bonilla F, Chinen J, Chinn IK, Dorsey M, et al. Update on the use of immunoglobulin in human disease: a review of evidence. J Allergy Clin Immunol. 2017;139:S1–S46. https://doi.org/10.1016/j.jaci.2016.09.023.

32. Vallat JM, Duchesne M, Corcia P, Richard L, Ghorab K, Magy L, et al. The wide spectrum of pathophysiologic mechanisms of paraproteinemic neuropathy. Neurology. 2021;96:214–25.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Hayashi T, Hayashi A, Fujimura Y, Masaki M, Kishikawa T, Sakaguchi H, et al. Dual preparation of plasma and platelet concentrates in platelet additive solution from platelet concentrates in plasma using a novel filtration system. Vox Sang. 2021;1–9.

Received: 14 April 2021 | Revised: 20 May 2021 | Accepted: 22 May 2021

DOI: 10.1111/vox.13164

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

International survey of strategies to mitigate transfusion-transmitted *Trypanosoma cruzi* in non-endemic countries, 2016–2018

Lauren A. Crowder[1] | Silvano Wendel[2] | Evan M. Bloch[3] | Sheila F. O'Brien[4] | Gilles Delage[5] | Silvia Sauleda[6] | David A. Leiby[7] | for the WP-TTID Subgroup on Parasites

[1]American Red Cross, Scientific Affairs, Rockville, Maryland, USA

[2]Blood Bank, Hospital Sirio Libanês, São Paulo, Brazil

[3]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[4]Epidemiology and Surveillance, Canadian Blood Services, Ottawa, Ontario, Canada

[5]Medical Affairs – Microbiology, Héma-Québec, Montreal, Quebec, Canada

[6]Transfusion Safety Laboratory, Catalonian Blood Bank (Banc de Sang i Teixits de Catalunya), Barcelona, Spain

[7]US Food and Drug Administration, CBER/OBRR, Silver Spring, Maryland, USA

Correspondence
David A. Leiby, Division of Emerging and Transfusion Transmitted Diseases, FDA/CBER/OBRR, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA.
Email: david.leiby@verizon.net

Funding information
None

Abstract

Background and Objectives: Chagas disease, caused by Trypanosoma cruzi, is endemic to Mexico, Central and South America. While initially limited to the Americas, emigration of infected persons triggered geographically broader blood safety challenges. To mitigate transfusion-transmitted Chagas (TTC), transfusion services implemented approaches including risk factor questions and serologic testing. We sought to understand and compare strategies in non-endemic countries.

Materials and Methods: Transfusion services in International Society of Blood Transfusion (ISBT)-affiliated organizations and members of the ISBT Working Party on Transfusion-Transmitted Infectious Diseases were invited to complete an online survey on T. cruzi mitigation strategies. The survey queried about cases of TTC, risk factors, testing methodology, educational materials, pathogen reduction, donor/product management, donor deferral and perceived public health concerns surrounding TTC.

Results: Responses were received from 27 institutions in 22 countries. Most countries (77.3%) reported no historical TTC cases, while 18.2% reported 1–5 cases and 4.5% reported 6–10 cases. Concern about Chagas among the general public and public health authorities was low, but 12 of 25 blood centres reported moderate/high concern. Overall, 17 countries mitigated for TTC: 15 used risk factor questions and 10tested for T. cruzi antibodies. Ten countries used pathogen reduction but not specifically to prevent TTC.

Conclusion: While Chagas is rarely cited as a public health concern, blood centres in many non-endemic countries, including those outside the Americas, implemented measures to mitigate risk. Mitigation focussed on risk factors associated with Latin American immigrants and serologic testing. Thus, despite the rarity of TTC, many non-endemic countries continue to address it as an ongoing blood safety risk.

KEYWORDS
blood transfusion, Chagas, mitigation, non-endemic, transmission, Trypanosoma cruzi

INTRODUCTION

Chagas disease or American Trypanosomiasis is caused by the protozoan parasite *Trypanosoma cruzi*. Most infected persons experience mild disease with intermittent parasitaemia and persistent antibody titres, while approximately 20%–30% of chronically infected persons will develop clinical Chagas disease including cardiac and gastrointestinal complications that may prove fatal [1, 2]. While vectoral or natural transmission is the most common route of infection in endemic areas of the Americas, human infections also occur congenitally, via oral ingestion and following laboratory accidents, organ transplantation or blood transfusion [3].

Transfusion-transmitted Chagas (TTC) was initially described in the endemic countries of Latin America, particularly Brazil, where hundreds of cases have been reported [4]. During the last 40 years, emigration of *T. cruzi*–infected persons from endemic to non-endemic countries of the world, especially those in North America and Europe [5, 6], has raised blood safety concerns across broad geographical areas. Addressing Chagas in a non-endemic country, much like malaria, poses potential challenges including identifying who is at-risk, the potential loss of blood donors if deferrals are implemented, the design of the most cost-effective testing strategy and considerations of pathogen reduction, if indeed mitigation strategies are deemed necessary. Currently, there is no universal approach for mitigating the risk of TTC in non-endemic countries.

In 2019, the Working Party on Transfusion-Transmitted Infectious Diseases (WP-TTID) of the International Society of Blood Transfusion (ISBT) published the results of a global survey examining the impact of parasitic infections [7]. Historical cases of TTC were reported from Brazil, Canada, Spain and the United States, while mitigation strategies varied from none to more stringent approaches (e.g., screening questions, testing, etc.) primarily in endemic countries. The WP-TTID sought to extend these observations by performing a more extensive and in-depth survey investigating the impact of *T. cruzi* on blood safety in non-endemic countries, especially those in North America and Europe.

METHODS

Survey development

The survey was developed by the ISBT WP-TTID Subgroup on Parasites (SoP) in consultation with a colleague (LAC) specialized in survey administration and analysis. Questions were developed by SoP experts in transfusion-transmitted parasites, blood donation testing and donor screening protocols. A cover page was sent with the survey to each recipient of the invitation making them aware of the types of questions that would be asked. This was done to aid respondents in knowing the necessary information to gather in preparation for answering the survey. Questions were organized into categories including population risk estimates, cases of TTC, risk factor screening, donor and donation testing methodologies, educational materials for donors, pathogen inactivation and reduction technologies, donor and product management protocols, donor deferral strategies and perceived public health concerns surrounding TTC (see DataS1).

Survey administration

Transfusion services in ISBT-affiliated organizations and members of the ISBT WP-TTID ($n = 52$) were contacted electronically in September 2019 and invited to complete an online survey on TTC mitigation strategies being used by their blood organizations. Responses were not anonymized so that duplicate responses from countries could be combined, if appropriate. The survey was administered using SurveyMonkey (SurveyMonkey Inc., San Mateo, CA).

Data analysis

Responses with no usable data provided were excluded. Duplicate responses from a specific country were either removed or combined, as appropriate, and follow-up of unclear responses was resolved through contacting the survey respondent directly. Data management and all analyses were carried out using SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY).

RESULTS

Twenty-nine blood centres responded to the survey and 27 provided usable data for this report. The 27 respondents represented national (63.0%), regional (18.5%) and single city or town (18.5%) blood centres in 22 countries. In aggregate, respondents reported over 22 million immigrants in their service areas primarily from Argentina, Brazil, Colombia and Mexico but included other Latin American countries.

Cases of transfusion-transmitted Chagas disease

Most (77.3%) countries reported no historical cases of TTC, although 18.2% reported 1–5 cases and 4.5% reported 6–10 cases (Table 1). Several of these cases have been detailed in published reports provided by the respondents [8–12], including the most recent TTC cases reported by the United States (2013) and Switzerland (2016) that were identified through lookback investigations [11, 13]. Of the reported TTC cases, three cases led to recipient death.

Concerns surrounding transfusion-transmitted Chagas

In general, concern about TTC was low, with over 50% of each sector expressing low, very low or absent risk, as reported by blood centres (Table 2). The highest concern was seen by blood centres and public health authorities in Europe. There was also perceived moderate

TABLE 1 Countries who responded to the survey and their reported historic cases of transfusion-transmitted Chagas (TTC)

Country	Historical cases of TTC
Australia	0
Austria	0
Canada[a]	1–5
China[a]	0
Denmark	0
France	0
Germany	0
Ghana	0
Japan	0
Luxembourg	0
Malaysia	0
Netherlands	0
New Zealand	0
Norway	0
Pakistan	0
Portugal	0
Russia	0
South Africa	0
Spain	1–5
Switzerland[a]	1–5
United Kingdom[a]	0
United States[a]	6–10

[a]Data received from two responding blood collection agencies.

TABLE 2 Concern about transfusion-transmitted Chagas from the general public, public health authorities and blood centres, as reported by blood centres

Level of concern	General public	Public health authorities	Blood centres
Absent	40.7%	14.8%	11.1%
Very low	40.7%	37.0%	28.0%
Low	7.4%	22.2%	11.1%
Moderate	3.7%	7.4%	25.9%
High	0%	11.1%	18.5%

Note: Two blood centres did not answer this question, total $n = 25$.

concern by the general public in one European country (Switzerland); however, this country did not report a high number of Latin American immigrants or areas with a high concentration of immigrants.

Risk factor screening

Asking donors about risk factors for Chagas remains one of the least expensive measures to prevent TTC. Fifteen countries (68%) indicated that they currently use risk factor questions during donor screening, primarily focussed on travel, country of birth, mother and grandmother's country of birth and residence in an endemic country (Table 3). Many respondents were unable to break down the number of donor deferrals into specific categories, so the number of deferrals due to these risk factor questions will not be reported here. Risk factor questions were reportedly used for permanent deferral in eight countries, short-term deferral in seven countries and selection of donors for testing in 10 countries. Three countries have a specific algorithm that is used to either permanently or temporarily defer or flag donors for testing based on the donor's answers to the risk factor screening questions.

TABLE 3 Risk factor questions asked to donors in respondent countries to screen for risk of Chagas

Risk factor question	Number of countries using this screening question
Birth in an endemic country	12
Travel to an endemic country	11[a]
Living in an endemic country	10
Mother or grandmother having been born in an endemic country	8
History of Chagas disease	8[b]
Living in substandard housing	2
History of blood transfusion in an endemic country	2
Exposure to the vector of Chagas disease (i.e., kissing bug)	1

[a]One country asks a broad question about travel abroad, which will capture travel to an endemic country.
[b]One country asks about history of unusual infections, which will capture Chagas disease.

Donation testing

Ten (45.5%) countries (Canada, France, Japan, Luxembourg, New Zealand, Portugal, Spain, Switzerland, Scotland and the United States) test for *T. cruzi* in their donor populations. The majority (80%) of countries use a commercially available chemiluminescent immunoassay (ChLIA) for their protocols. Half of the countries with testing use enzyme-linked immunosorbent assay (ELISA) for their testing protocols, with three countries (France, Spain and the United States) reportedly using both methods. Less common testing methodologies – of which some may be confirmatory/supplemental testing – include enzyme immunoassay (EIA; four countries), indirect fluorescent antibody (IFA; four countries), enzyme strip assay (ESA; three countries), nucleic acid test (NAT; one country) and western blot (WB; one country). No countries reported using indirect haemagglutination assay or radioimmunoprecipitation assay testing methodologies to screen donations for *T. cruzi*.

Product manipulation: Leukoreduction and pathogen inactivation/pathogen reduction

Ten countries are currently using leukoreduction; nine countries leukoreducing 100% of their products and one country leukoreducing 86% of products. Platelets and plasma in seven countries are pathogen-reduced or inactivated, but this strategy was not designed specifically to mitigate risk of TTC. Four countries (France, Portugal, Switzerland and the United States) are currently using the Intercept System, and the other three use methylene blue for plasma (Spain), Mirasol for platelets and solvent detergent for plasma (Luxembourg) and Octaplas for certain recipients (Scotland). Luxembourg and Switzerland are the only two countries who responded that they use PI/PR for 100% of eligible blood products. Portugal, Scotland, France and the United States, each reports using PI/PR but for less than 25% of blood products.

Donor and product management protocols

Positive donors in 12 different blood centres representing 10 countries used letter (nine centres), telephone (six centres), face-to-face (four centres) or email (two centres) to inform donors about their positive test results. Six blood centres used multiple modes to contact positive donors. Nine of these blood centres provide counselling to the positive donors, 11 centres refer positive donors to a physician for treatment and all 12 blood centres defer these positive donors from future donations. Eleven centres retrieve in-date components where they are either discarded or used for research. All 12 centres initiate lookback investigations; however, the period of lookback varied widely from centre to centre based on testing or other risk factors.

Donor deferral and education

Donors with a history of Chagas are deferred permanently from donating in 16 countries. Donors with risk factors for Chagas or *T. cruzi* infection are deferred in 12 countries ranging from 1 month to a permanent deferral, depending on the risk factor. Donors with a positive test result are deferred permanently in 11 countries and for 3 years in one country (France), with re-entry permitted in four countries (France, Japan, the United Kingdom and the United States).

Of the 22 countries who responded to the survey and the 10 countries who test donations, only two provide educational materials regarding Chagas, *T. cruzi*, testing and/or treatment to their donors prior to testing. These two countries, the United Kingdom (Scotland) and Canada, provide reading materials, pamphlets or other similar products. Canada provides these materials in English and French; Scotland provides these materials in English and Spanish or Portuguese. Scotland additionally provides verbal counselling by collection staff and social media, video or other multimedia educational products for their donors.

DISCUSSION

The first case of TTC was reported in Brazil in 1952 [14]. Thereafter, Chagas was recognized as a blood safety threat in much of South America and later in the rest of Latin America where *T. cruzi* is endemic. In the last 50 years, waves of migration from Latin America to North America and later Europe occurred due to civil unrest and socioeconomic issues [1, 15, 16]; some of these at-risk individuals eventually chose to become blood donors. Thereafter, blood safety concerns were raised as TTC cases began to appear in non-endemic countries, albeit infrequently. The United States reported its first case in 1987, Canada in 1989 and European countries a few decades later (e.g., Spain 2007/2008) [9, 17–19]. In response to TTC cases, non-endemic countries began to implement mitigation strategies that varied greatly and were country specific. The present survey by the SoP sought to better understand these strategies, including their similarities and differences.

The survey was sent electronically to blood collection organizations in non-endemic countries from the membership rolls of the WP-TTID and by ISBT to member organizations. Unfortunately, we do not know distribution numbers for the latter group, so the overall response rate could not be determined. However, responses received represented a wide geographical spread, with particular representation by North America and Europe where our survey suggested historical cases of TTC had been reported. Of note, survey results were received from Japan, which has had longstanding travel to and from Brazil where a large Japanese population resides [20].

As in our broader survey of transfusion-transmitted parasitic infections [7], public health concerns were assessed by survey respondents for their respective countries. In most cases, the general public's concern regarding Chagas was low to absent. This is perhaps not surprising as outside of Latin America, Chagas is likely perceived as an exotic, not a well-understood disease with virtually no direct impact to people in non-endemic countries. Similarly, for most public health officials, Chagas has relatively little impact as it is not an overwhelming public health problem due to the absence of vector-borne transmission. The exception among our respondents may be the United States, due to its proximity to endemic countries and reports of vector-derived transmission of *T. cruzi* [21]. In contrast, blood centres in non-endemic countries reported higher levels of concern since TTC has been shown to be a risk for blood recipients, not only in North America but also in Europe.

The survey results suggested that mitigation strategies varied widely, especially when viewed in the context of immigrant populations and historical cases of TTC. In North America, with larger immigrant populations stemming from the proximity to Latin America, broader strategies were implemented. Upon approval of blood screening assays in 2006, the United States initially screened all blood donors for *T. cruzi* antibodies due to relatively high seroprevalence rates in portions of the country and eight historical cases of TTC [22]. Subsequent studies indicated an absence of incident cases [23], thus in 2010, the testing algorithm was changed to one time testing of all donors [24, 25]. Canada, with fewer Latin American immigrants, but three historical cases of TTC, uses a selective approach, testing only donors demonstrating risk. In contrast, European countries with

smaller immigrant populations and the absence of historical transfusion cases often choose to use screening questions, followed by deferral based on risk. An exception is Spain, with a comparatively larger population of immigrants from endemic areas than the rest of Europe and at least five historical cases of TTC, that uses a strategy similar to Canada's: selective testing based on risk following a 4-month deferral since the donor's last visit to an endemic area.

For many non-endemic countries where overall risk of TTC is low, mitigation was accomplished by risk factor screening questions alone. In particular, birth in an endemic country by the donor or the donor's mother/maternal grandmother resulted in deferral of the donor. While the use of these questions may not be the most sensitive approach, for countries with small at-risk populations, this approach likely has minimal effect on blood availability. Further, the use of risk factor questions and subsequent deferral for a limited donor population is likely more cost-effective compared to molecular or serologic testing strategies.

When countries chose to screen blood donors by testing, either outright or following selection based on risk factor questions, serologic assays were used. In non-endemic countries where vector-borne transmission does not occur, serologic assays have been shown to be highly effective at identifying antibody-positive donors who may be parasitaemic following infection in their country of birth by vectorial or congenital transmission [26]. In general, infected donors maintain lifelong antibody titres readily detected by serologic assays, thereby allowing for identification and deferral of infected donors who may have intermittent parasitaemia. Survey results suggested that the serologic assays of choice in non-endemic countries were ELISA/EIA formats that routinely demonstrate extremely high sensitivity and specificity.

The survey also revealed the use of product manipulation to reduce the risk of TTC, specifically leukoreduction and pathogen inactivation. However, in no case was either of these technologies implemented explicitly to mitigate the risk of TTC. Leukoreduction has been shown to reduce but not eliminate the *T. cruzi* parasite from blood components [27], while pathogen inactivation has been shown to be effective against *T. cruzi* in plasma and platelet products [28, 29], the latter accounting for most cases of TTC [30]. Although rare, TTC cases attributed to red cells have occurred [31], thus the absence of pathogen reduction for red cells to date represents a limitation of this technology.

This study had several key strengths, including its ability to reach an international audience and receive and analyse information from these blood centres in a quick manner. The survey asked a variety of questions to assess mitigation efforts from various countries, and the aggregate results can provide transparency to the efforts utilized in non-endemic areas. However, as with any survey-based research study, several limitations must be considered when interpreting the results. The response rate of this survey was low, with only 27 responses representing 22 distinct non-endemic countries; however, evaluating the exact response rate was further complicated by distribution to ISBT member organizations resulting in a response rate that could not be determined for this group. The study was also limited by the generalizability of the results based on the countries who responded. Also, in several instances, more than one response was received from a country, but analysis of these responses was consistent in each case. Another limitation was that the survey asked respondents to estimate the level of concern around TTC of the public, blood collectors and public health authorities. While the respondents likely knew the opinions of their own blood centres, respondents were left to make estimations of the other two groups, which may have not been accurate. The last limitation was selection bias. While we sought to receive responses from a broad range of *T. cruzi* non-endemic countries, we were limited by survey distribution constraints including the memberships roles of the WP-TTID, which often represent resource-rich countries.

In summary, despite minimal concern by the general population or public health officials in *T. cruzi* non-endemic countries, many blood centres use various mitigation strategies to minimize the risk of TTC. The approach used to mitigate risk is in part dictated by the size of the at-risk population, history of TTC, cost-effectiveness and potential donor loss through deferrals. TTC remains a relatively low risk in non-endemic countries, but mitigation strategies have contributed to further reduction in risk and the absence of extant cases of transmission by blood transfusion.

ACKNOWLEDGEMENTS

All authors have contributed equally to the development and desgin of the survey, analysis of the data, and the drafting, reveiw and approval of the manuscript. The authors are members of the Subgroup on Parasites of the Working Party on Transfusion-Transmitted Infectious Diseases of the International Society of Blood Transfusion. The authors wish to thank the following transfusion medicine leaders who completed the survey, listed by the country (alphabetically) and institution they represent: Australia, Helen Faddy & Clive Seed, Australian Red Cross Blood Service; Austria, Christian Gabriel, Ludwig Boltzmann Institute; Canada, Sheila O'Brien, Canadian Blood Services; Canada, Gilles Delage, Héma-Québec; China, Hui Ping, Macao Blood Transfusion Services; China, Wai-Chiu Tsoi, Hong Kong Red Cross Blood Transfusion Service; Denmark, Lene Holm Harritshoj, Gentofte Hospital; France, Sophie Le Cam, EtablissementFrançais du Sang; Germany, Erhard Seifried, Blood Transfusion Centre of the German Red Cross; Ghana, Mavis Okyere, National Blood Service; Japan, Masahiro Satake, Japanese Red Cross Central Blood Institute; Luxembourg, Anne Schuhmacher&AndréeHeinricy, Red Cross of Luxemburg; Malaysia, FaraizahAbdKarim, Hospital Ampang; Netherlands, Mariel Casparie, Sanquin; New Zealand, Peter Flanagan, New Zealand Blood Service; Norway, LiseSofieNissen-Meyer, Oslo University Hospital; Pakistan, Hasan Abbas Zaheer, Ministry of Health, Government of Pakistan; Portugal, Mario Chin Muon, IstitutoPortugues do Sangue e da Transplantação; Russia, Eugene Zhiburt, Pirogov National Medical Surgical Center; South Africa, Marion Vermeulen, South African National Blood Service & Western Cape Blood Service; Spain, Silvia Sauleda, Banc de Sang Teixits Catalonia; Switzerland, TizianaJanner, Transfusion SRC Switzerland; Switzerland, ChristophNiederhauser, Interregional Blood Transfusion SRC; United Kingdom, Stuart Blackmore, Welsh Blood Service; United Kingdom, Lisa Jarvis, Scottish National Blood Transfusion Service; United States, Jamel Groves & Susan Stramer, American Red Cross; and United States, Michael Busch & Brian Custer, Vitalant.

CONFLICT OF INTEREST

There are no conflicts of interest. This work reflects the views of the authors and should not be construed to represent FDA's views or policies.

ORCID

Silvano Wendel https://orcid.org/0000-0002-1941-7733
Evan M. Bloch https://orcid.org/0000-0001-8181-9517
Sheila F. O'Brien https://orcid.org/0000-0002-5332-2789
David A. Leiby https://orcid.org/0000-0002-0596-7160

REFERENCES

1. Perez-Molina JA, Molina I. Chagas disease. Lancet. 2018;391:82–94.
2. Bern C, Messenger LA, Whitman JD, Maguire JH. Chagas disease in the United States: a public health approach. ClinMicrobiol Rev. 2020;33:e00023-19.
3. Bern C. Chagas' disease. New Eng J Med. 2015;373:1882.
4. Wendel S, Gonzaga AL. Chagas' disease and blood transfusion: a New World problem? Vox Sang. 1993;64:1–12.
5. Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the burden of Chagas disease in the United States. PLoS Negl Trop Dis. 2016;10:e0005033.
6. Requena-Mendez A, Aldasoro E, de Lazzari E, Sicuri E, Brown M, Moore DAJ, et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2015;9:e0003540.
7. Leiby DA, O'Brien SF, Wendel S, Nguyen ML, Delage G, Devare SG, et al. International survey on the impact of parasitic infections: frequency of transmission and current mitigation strategies. Vox Sang. 2019;114:17–27.
8. Fearon MA, Scalia V, Huang M, Dines I, Ndao M, Lagace-Wiens P. A case of vertical transmission of Chagas disease contracted via blood transfusion in Canada. Can J Infect Dis Med Microbiol. 2013;24:32–4.
9. Nickerson P, Orr P, Schroeder ML, Sekla L, Johnston JB. Transfusion-associated *Trypanosoma cruzi* infection in a non-endemic area. Ann Intern Med. 1989;111:851–3.
10. Lane DJ, Sher G, Ward B, Ndao M, Leiby DA, Hewlett B. Investigation of the second case of transfusion transmitted Chagas diseases in Canada. Blood. 2000;96:60a [abstract].
11. Ries J, Komarek A, Gottschalk J, Brand B, Amsler L, Jutzi M, et al. A case of possible Chagas transmission by blood transfusion in Switzerland. Transfus Med Hemother. 2016;43:415–7.
12. Niederhauser C, Gottschalk J, Tinguely C. Selective testing of at-risk blood donors for *Trypanosoma cruzi* and *Plasmodium* spp. in Switzerland. Transfus Med Hemother. 2016;43:169–76.
13. Kessler DA, Shi PA, Avecilla ST, Shaz BH. Results of lookback for Chagas disease since the inception of donor screening at New York blood center. Transfusion. 2013;53:1083–7.
14. Freitas JLP, Veto AN, Sonntag R, Biancalana A, Nussenzweig V, Barreto JG. As primeiras verificações de transmissão acidental da moléstia de Chagas ao homem por transfusão de sangue. Rev Paul Med. 1952;40:36–40.
15. Angheben A, Boix L, Buonfrate D, Gobbi F, Bisoffi Z, Pupella S, et al. Chagas disease and transfusion medicine: a perspective from non-endemic countries. Blood Transfus. 2015;13:540–50.
16. Leiby DA, Herron RM Jr, Read EJ, Lenes BA, Stumpf RJ. *Trypanosoma cruzi* in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. Transfusion. 2002;42:549–55.
17. Geiseler PJ, Ito JI, Tegtmeier BR, Kernt PR. Fulminant Chagas disease (CD) in bone marrow transplantation (BMT). 27th ICAAC 1987;(Abstract 418):169.
18. Abalo M, Adelantado M, Areal C, Castrillo A, Castro A, Cid J, et al. Tracing of one year of Chagas screening at the Centro de transfusion de Galicia (C.T.G.). concerning a positive blood donor. Vox Sang. 2007;93:140 Abstract P-235.
19. Flores-Chavez M, Fernandez B, Puente S, Torres P, Rodriguez M, Monedero C, et al. Transfusionalchagas disease: parasitological and serological monitoring of an infected recipient and blood donor. Clin Infect Dis. 2008;46:e44-7.
20. Sayama Y, Furui Y, Takakura A, Ishinoda M, Matsumoto C, Taira R, et al. Seroprevalence of *Trypanosoma cruzi* infection among at-risk blood donors in Japan. Transfusion. 2019;59:287–94.
21. Montgomery SP, Parise ME, Dotson EM, Bialek SR. What do we know about Chagas disease in the United States? Am J Top Med Hyg. 2016;95:1225–7.
22. Bern C, Montgomery SP, Katz L, Caglioti S, Stramer SL. Chagas disease and the US blood supply. Curr Opin Infect Dis. 2008;21:476–82.
23. Stramer SL, Notari EP, Townsend RL, Custer B, Kamel H, Busch MP, et al. No evidence of *Trypanosoma cruzi* incidence in United States blood donors: a 4-year study. Vox Sang. 2012;103:58 [abstract].
24. Dodd RY, Groves JA, Townsend RL, Notari EP, Foster GA, Custer B, et al. Impact of one-time testing for *Trypanosoma cruzi* antibodies among blood donors in the United States. Transfusion. 2019;59:1016–23.
25. US Food and Drug Administration, Center for Biologics Evaluation and Research. Use of serological tests to reduce the risk of transmission of *Trypanosoma cruzi* infection in blood and blood components. December 2017 [cited March 18, 2021]. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM528600.pdf
26. Leiby DA, Nguyen ML, Proctor MC, Townsend RL, Stramer SL. Frequency of *Trypanosoma cruzi* parasitemia among infected blood donors with a potential association between parasite lineage and transfusion transmission. Transfusion. 2017;57:1426–32.
27. Jimenez-Marco T, Cancino-Faure B, Girona-Llobera E, Alcover MM, Riera C, Fisa R. The effectiveness of riboflavin and ultraviolet light pathogen reduction technology in eliminating *Trypanosoma cruzi* from leukoreduced whole blood. Transfusion. 2017;57:1440–7.
28. Cardo LJ, Salata J, Mendez J, Reddy H, Goodrich R. Pathogen inactivation of *Trypanosoma cruzi* in plasma and platelet concentrates using riboflavin and ultraviolet light. Transfus Apher Sci. 2007;37:131–7.
29. Castro E, Girones N, Bueno JL, Carrion J, Lin L, Fresno M. The efficacy of photochemical treatment with amotosalen HCl and ultraviolet a (INTERCEPT) for inactivation of *Trypanosoma cruzi* in pooled buffy-coat platelets. Transfusion. 2007;47:434–41.
30. Benjamin RJ, Stramer SL, Leiby DA, Dodd RY, Fearon M, Castro E. *Trypanosoma cruzi* infection in North America and Spain: evidence in support of transfusion transmission. Transfusion. 2012;52:1913–21.
31. Blumental S, Lambermont M, Heijmans C, Rodenbach MP, el Kenz H, Sondag D, et al. First documented transmission of *Trypanosoma cruzi* infection through blood transfusion in a child with sickle-cell disease in Belgium. PLoS Negl Trop Dis. 2015;9:e0003986.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Crowder LA, Wendel S, Bloch EM, et al. International survey of strategies to mitigate transfusion-transmitted *Trypanosoma cruzi* in non-endemic countries, 2016–2018. Vox Sang. 2021;1–6.

Received: 3 January 2021 | Revised: 14 April 2021 | Accepted: 14 April 2021

DOI: 10.1111/vox.13125

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Effect of red blood cell transfusion on inflammation, endothelial cell activation and coagulation in the critically ill

Lisa van Manen[1,2] | Maike E. van Hezel[1,2] | Margit Boshuizen[1,2] | Marleen Straat[1] | Angelique M. E. de Man[3] | Charlotte Dekimpe[4] | Karen Vanhoorelbeke[4] | Robin van Bruggen[2] | Nicole P. Juffermans[1,5]

[1]Department of Intensive Care Medicine and Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam UMC, Location VUmc, University of Amsterdam, Amsterdam, The Netherlands

[2]Department of Blood Cell Research, Sanquin research and Landsteiner Laboratory, University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Intensive Care Medicine, Amsterdam UMC, Location VUmc, Amsterdam, The Netherlands

[4]Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium

[5]Department of Intensive Care Medicine, OLVG Hospital, Amsterdam, The Netherlands

Correspondence
Lisa van Manen, Department of Intensive Care Medicine and Laboratory of Experimental Intensive Care and Anesthesiology, University of Amsterdam, Amsterdam UMC, location AMC, Amsterdam, The Netherlands.
Email: l.vanmanen@amsterdamumc.nl

Abstract

Background and Objectives: Red blood cell (RBC) transfusion is a frequently applied intervention in an intensive care unit. However, transfusion is associated with adverse outcomes including organ failure and thrombo-embolic events. Mechanisms of these effects are not known but may be related to activation of the endothelium or of the coagulation or inflammatory system. We hypothesized that a RBC transfusion in the critically ill would result in further activation of these systems.

Materials and Methods: In 74 non-bleeding critically ill patients receiving one RBC unit, markers of inflammation, endothelial cell activation and coagulation were measured before transfusion, at 1 h after transfusion and 24 h after transfusion. The impact of disease severity of the recipient on these changes was assessed by comparing septic and non-septic patients (according to sepsis-3 definition) and by correlation of biomarkers with the sequential organ failure assessment (SOFA) score.

Results: Levels of von Willebrand Factor (vWF), soluble ICAM-1, soluble thrombomodulin, fibrinogen and d-dimer were already high at baseline, whereas ADAMTS13 levels were low. VWF levels increased significantly 24 h after RBC transfusion (median 478% (338–597) vs. 526% (395–623), $p = 0.009$). The other biomarkers did not change significantly. Post transfusion change was not dependent on the presence of sepsis and was not correlated with SOFA score.

Conclusion: RBC transfusion in critically ill patients was associated with an increase in circulating vWF levels, suggesting a further increase in activation of the endothelium, a finding that was independent of the presence of sepsis or organ injury level.

KEYWORDS
coagulation, critically ill patients, endothelial activation, inflammation, RBC transfusion

Robin van Bruggen and Nicole P. Juffermans contributed equally to this work.

INTRODUCTION

More than 1 out of four patients in an intensive care unit (ICU) receive a red blood cell (RBC) transfusion during their admission, rendering RBC transfusion one of the most frequently applied interventions at the ICU [1]. However, RBC transfusion is associated with adverse outcomes including organ failure and thrombo-embolic events, in particular in the critically ill [2–5]. The mechanisms responsible for these adverse events are not fully understood.

Critically ill patients are often in an inflammatory state in which the endothelium and coagulation system are already activated before transfusion [6]. The presence of an inflammatory state in the recipient has been shown to be a risk factor for the development of transfusion related acute lung injury (TRALI) [5]. Also, in patients with an inflammatory state that do not develop full-blown TRALI, an extra 'hit' by the RBC transfusion may exacerbate inflammation, potentially resulting in transfusion related adverse events [7–10]. Since the vascular endothelium and glycocalyx are among the first that interact with the donor RBCs after transfusion, these structures might also play a role in the pathophysiology. RBC transfusion is associated with increased biomarker levels of endothelial cell activation in haematological and paediatric patients [11, 12]. Endothelial cell activation can lead to increased endothelial permeability with neutrophil extravasation and capillary leakage, resulting in organ injury [13]. Activation of the endothelium also activates the coagulation system, resulting in (micro)thrombus formation [14, 15]. Therefore, the vascular endothelium and activation of the coagulation system might be involved in the adverse events of RBC transfusion.

The aim of this study is to investigate the effect of RBC transfusion on several biomarkers of inflammation, endothelial cell activation and coagulation in adult critically ill patients (Table 1). We hypothesized that a RBC transfusion in the critically ill results in further activation of the vascular endothelium and also in activation of the coagulation system. Since sepsis is a risk factor for developing transfusion- associated adverse events, we expected a greater effect in septic patient compared to non-septic patients [5].

TABLE 1 Measured biomarkers and their function

Biomarker	Function
Markers of inflammation	
IL-6	Pro-inflammatory cytokine
TNF-alpha	Pro-inflammatory cytokine
Markers of endothelial cell activation	
Soluble syndecan-1	Marker of glycocalyx degradation
Soluble thrombomodulin	Marker of endothelial cell damage
Soluble ICAM-1	Marker of endothelial cell activation
Von Willebrand Factor	Marker of endothelial ceactivation and involved in coagulation cascade
ADAMTS13	Responsible for cleavage of ultra large multimers of vWF
Markers of coagulation	
D-dimer	Fibrin degradation product
Fibrinogen	Precursor of fibrin, converted into fibrin during clot formation

Abbreviations: ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukine-6; TNF, tumour necrosis factor.

MATERIALS AND METHODS

Study design

A prospective, observational study was conducted on the intensive care units of two tertiary hospitals in the Netherlands between 2011–2015 and 2017–2018. Due to logistical problems the study was temporarily interrupted between 2015 and 2017. The study was ethically approved by the medical ethical committee of the Amsterdam University Medical Centre (NTR 6596; NL61833.018.17). Written informed consent was obtained from all participants or their legal representatives. Non-bleeding patients receiving one RBC unit to correct for anaemia were included. Patients without an indwelling arterial catheter were excluded. Patients were lost to follow-up when indwelling arterial catheter was removed. Local transfusion protocol dictated a transfusion trigger of 7.0 g/dl in the general ICU population and 8.0 g/dl for those with acute coronary syndrome. The RBC unit was plasma reduced, leucocyte depleted and stored in additive solution (SAGM). The average volume of RBC unit is 270–290 ml. The RBC unit was produced according to the national standards of Sanquin Blood Supply Foundation, Amsterdam, the Netherlands. Sepsis was defined as a sequential organ failure assessment (SOFA) score of at least 2 and a suspected or proven infection treated with antibiotics according to the Sepsis-3 criteria [16].

Study procedures

Blood was drawn from an indwelling arterial catheter into EDTA and citrate tubes before transfusion, within 1 h after the completion of the transfusion and 24 and 48 h after transfusion. Since we expected the effect of the RBC transfusion within 24 h after RBC transfusion we measured biomarkers directly after and 24 h after transfusion. We did not expect an effect of transfusion after 48 h, therefore we used this sample only to measure the vWF antigen. A further increase after 48 h could indicate a natural upward course of vWF during ICU admission.

A complete blood count was done using EDTA blood (Sysmex XN9000, Etten-Leur, The Netherlands). Thereafter blood was centrifuged for 20 min at 1500G. Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and d-dimer levels were determined in citrate plasma (CS2500, Siemens Healthcare GmbH, Germany). Plasma samples were frozen at −80°C until further analysis.

Measured biomarkers are listed in Table 1. Soluble Syndecan-1, soluble ICAM-1, soluble thrombomodulin (TM), IL-6 and TNF-alpha

were measured in EDTA plasma with a custom designed human premixed multi-analyte luminex assay (Luminex R&D Systems Inc., Minneapolis, MN, USA) as described by the manufacturer. Von Willebrand Factor (vWF) antigen was measured in citrate plasma with ELISA (DAKO, Glostrup, Denmark).

Human ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) antigen levels were determined using a monoclonal antibody-based human ADAMTS13 antigen ELISA, as previously described [17–19]. Microtiter plates were coated with the monoclonal mouse anti-human ADAMTS13 antibody 3H9 [17, 18, 20] overnight at 4°C (5 μg/ml in carbonate/bicarbonate buffer) and subsequently blocked with phosphate-buffered saline (PBS) with 3% dried milk powder for 2 h at room temperature (RT). Next, plasma samples (starting dilution of 1/50) were added in a 1.5 over 2.5 dilution series and incubated for 1.5 h at 37°C. Captured ADAMTS13 was detected using a mixture of biotinylated mouse anti-human ADAMTS13 antibodies 17G2 and 19H4 [17, 18, 21] (1.5 μg/ml each, incubation for 1 h at RT) followed by horseradish peroxidase

TABLE 2 Baseline characteristics of 74 critically ill non-bleeding patients receiving a RBC transfusion

Characteristic	Total ($N = 74$)	No sepsis ($N = 33$)	Sepsis ($N = 41$)	*p*-value
Sex, male (#, %)	41 (55)	23 (69.7)	18 (44.0)	0.03
Age, years (median, IQR)	63 (57–73)	63 (57–72)	64 (57–75)	0.56
Surgical (#, %)	46 (62)	20 (61)	26 (63)	0.99
SOFA score at inclusion (median, IQR)	8.5 (7–11)	9 (7–10)	8 (7–11)	0.76
Haemoglobin level at inclusion (g/dl) (median, IQR)	6.8 (6.3–7.4)	6.8 (6.5–7.4)	6.6 (6.3–7.4)	0.80
Days at ICU at inclusion (median, IQR)	11 (4–17)	4 (2.8–15.0)	13.5 (7.3–19.5)	0.00
Hospital mortality (#, %)	18 (24.7)	5 (15.2)	14 (34.1)	0.11
Age of transfusion unit, days (median, IQR)	13 (7–22)	13 (6–22)	16 (8–21)	0.43

Note: Subgroups of septic and non-septic patients based on Sepsis-3 criteria.
Abbreviations: ICU, intensive care unit; SOFA, sequential organ assessment score.

TABLE 3 Level of biomarkers of endothelial cell activation and inflammation before transfusion, within hour after transfusion and 24 h after transfusion

Biomarker	Reference value	Before transfusion median (IQR)	1 h after transfusion median (IQR)	24 h after transfusion median (IQR)	*p*-value
vWF ag (%)	50%–150%	478 (338–597)	481 (348–614)	526 (395–623)	0.009
ADAMTS13 ag (%)	50%–150%	40.4 (31.8–53.6)	43.9 (31.7–54.7)	40.8 (31.4–52.2)	0.06
vWF/ADAMTS13 ratio		11.6 (7.2–18.0)	11.7 (7.0–18.1)	12.1 (8.4–17.2)	0.98
sICAM-1 (ng/ml)	60–218	462 (324–605)	448 (334–601)	453 (337–609)	0.83
sTM (ng/ml)	0.5–5.7	7.6 (5.9–11.3)	7.6 (5.4–11.2)	7.5 (5.2–11.1)	0.26
Syndecan-1 (ng/ml)	50–100	2.9 (2.3–3.6)	2.9 (2.3–3.7)	2.9 (2.1–3.7)	0.94
TNFa (pg/ml)	0–16	11.5 (9–15.3)	12 (8.7–16)	12.5 (9–14.8)	0.09
IL-6 (pg/ml)	5–15	46.2 (18.2–75.3)	43.3 (18.1–71.4)	35.2 (18.2–64.4)	0.32

Note: *p*-value based on Friedman test.
Abbreviations: ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; IL-6, interleukine-6; sICAM-1, soluble intercellular adhesion molecule 1; sTM, soluble thrombomodulin; TNF, tumour necrosis factor; vWF, von Willebrand factor.

TABLE 4 Level of coagulation markers before and 24 h after transfusion

Biomarker	Reference value	Before transfusion median (IQR)	24 h after transfusion median (IQR)	*p*-value
Platelets (10 ⁹/L)	150–400	186 (114–269)	199 (110–289)	0.9
D-dimer (mg/L)	0–0.5	5.54 (3.27–9.35)	4.63 (2.82–9.35)	0.45
Fibrinogen (mg/ml)	1.5–4.0	5 (4.35–6.65)	5.2 (4.6–6.9)	0.36
APTT (s)	30–40	34 (26–46.5)	30 (25–43)	0.08
PT (s)	9.5–13.5	11.6 (11.1–12.6)	11.6 (10.9–12.3)	0.35

Note: *p*-value based on Wilcoxon signed rank test.
Abbreviations: APTT, activated partial thromboplastin time; PT, prothrombin time.

(HRP)-labelled streptavidin (1/10,000; Roche Diagnostics, Mannheim, Germany) (incubation for 1 h at RT). The colorimetric reaction was initiated by addition of o-phenylenediamine (OPD) and H_2O_2, stopped with 4 M sulfuric acid, and the absorbance was measured at 490 nm. A dilution series of a normal human plasma pool (NHP, plasma from ≥20 healthy donors, set at 100%) was used as a reference, from which the ADAMTS13 antigen levels were interpolated.

Statistical analysis

Variables are presented as means with standard deviation or as medians with interquartile ranges when not normally distributed, and categorical data are presented as number with percentage. For continuous data, comparisons between two groups were made using the *t*-test or when not normally distributed the Mann–Whitney U test. More than two groups were compared using the Kruskal Wallis test. For categorical data, the Chi-squared test was used. Paired data analysis were done with the Friedman test and Wilcoxon signed rank test. Correlation coefficient between the delta of the biomarkers and SOFA score was calculated using the Spearman correlation. A P-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using R statistics (v 3.5.1).

RESULTS

Seventy-four patients (55% male) were included with an median age of 63 years old (IQR 57–73). In four patients, 24 h timepoint was missed because indwelling arterial catheter was removed. Patients had a SOFA score of 8.5 (IQR 7–11). Most patients came from surgical departments (62%). Forty-one patients (55%) fulfilled the sepsis criteria. Baseline characteristics are given in Table 2. Median haemoglobin level at inclusion was low (6.8 g/dl) and increased after transfusion (7.9 g/dl, $p < 0.001$). Septic patients were more often female and had a longer ICU admission duration at time of inclusion compared to non-septic patients.

RBC transfusion resulted in an increase in vWF antigen, but not in other markers

VWF antigen levels were high at baseline (median 478% [IQR 338–597]) and increased significantly 24 h after RBC transfusion (median 526% [IQR 395–623]; $p = 0.009$). VWF levels did not further increase 48 h after transfusion. Its cleaving protease ADAMTS13 was very low at baseline but was not affected by the transfusion. VWF/ADAMTS13 ratio was not affected by the transfusion. Levels of soluble ICAM-1 and soluble TM were high at baseline but did not change significantly after transfusion. Levels of soluble syndecan-1 were low and did not increase significantly after transfusion. Concentration of IL-6 and TNF-alpha did also not significantly change (Table 3).

D-dimer and fibrinogen levels were elevated at baseline, but did not increase after transfusion. Platelet count, PT and APTT were in between reference values and also did not significantly change after transfusion (Table 4).

The inflammatory state of the recipient did not impact the effect of a RBC transfusion

Septic patients had higher baseline levels of soluble Syndecan-1, vWF antigen and d-dimer compared to non-septic patients, whereas ADAMTS13 antigen levels were lower ($p < 0.05$). No differences were seen between the two groups in post transfusion change of markers of endothelial cell activation or coagulation (data not shown). In addition, post transfusion change of markers did not correlate with organ injury as assessed with the SOFA score (Figure 1).

DISCUSSION

This study investigated the effect of a RBC transfusion on several markers of inflammation, endothelial cell activation and coagulation in the critically ill patient. Before transfusion, levels of vWF antigen, sICAM-1 and sTM were already high, indicating that the vascular endothelium is activated in the critically ill patient [22]. D-dimer and fibrinogen levels were also elevated at baseline. We found that RBC transfusion is associated with an increase in circulating vWF antigen levels, independent of the presence of sepsis or organ injury level of the patient. The other biomarkers did not show a post transfusion change.

So far, only one study has studied the effect of RBC transfusion on vWF antigen levels. In cardiology patients, an increase in vWF antigen levels was not observed directly following RBC transfusion [23]. However, as vWF release takes some time following a stimulus this time point may have been too short after transfusion [24]. We think it is unlikely the increase of vWF is caused by vWF that was present in RBC units, since increase was not observed immediately after transfusion but after 24 h. Most likely, RBC transfusion led to shedding of vWF from endothelial cells.

vWF antigen plays an important role in arterial and venous thrombus formation [15, 25, 26]. The increase in circulating vWF antigen levels after RBC transfusion can therefore potentially explain the increase in thrombo-embolic events found after transfusion [4, 27]. However, we did not find an effect on markers of disseminated intravascular coagulation (DIC), such as a decreased platelet count, which is in line with earlier research [28]. Furthermore, the vWF/ADAMTS13 ratio did not increase. Therefore, the impact of 1 RBC unit on the development of thrombosis seems minimal. However, patients often receive multiple transfusions over time during their ICU stay. Whether multiple transfusions result in a more pronounced increase of vWF, followed by changes in DIC markers or the vWF/ADAMTS13 ratio should be investigated in a future study. The occurrence of thrombosis in critically ill patients is associated with

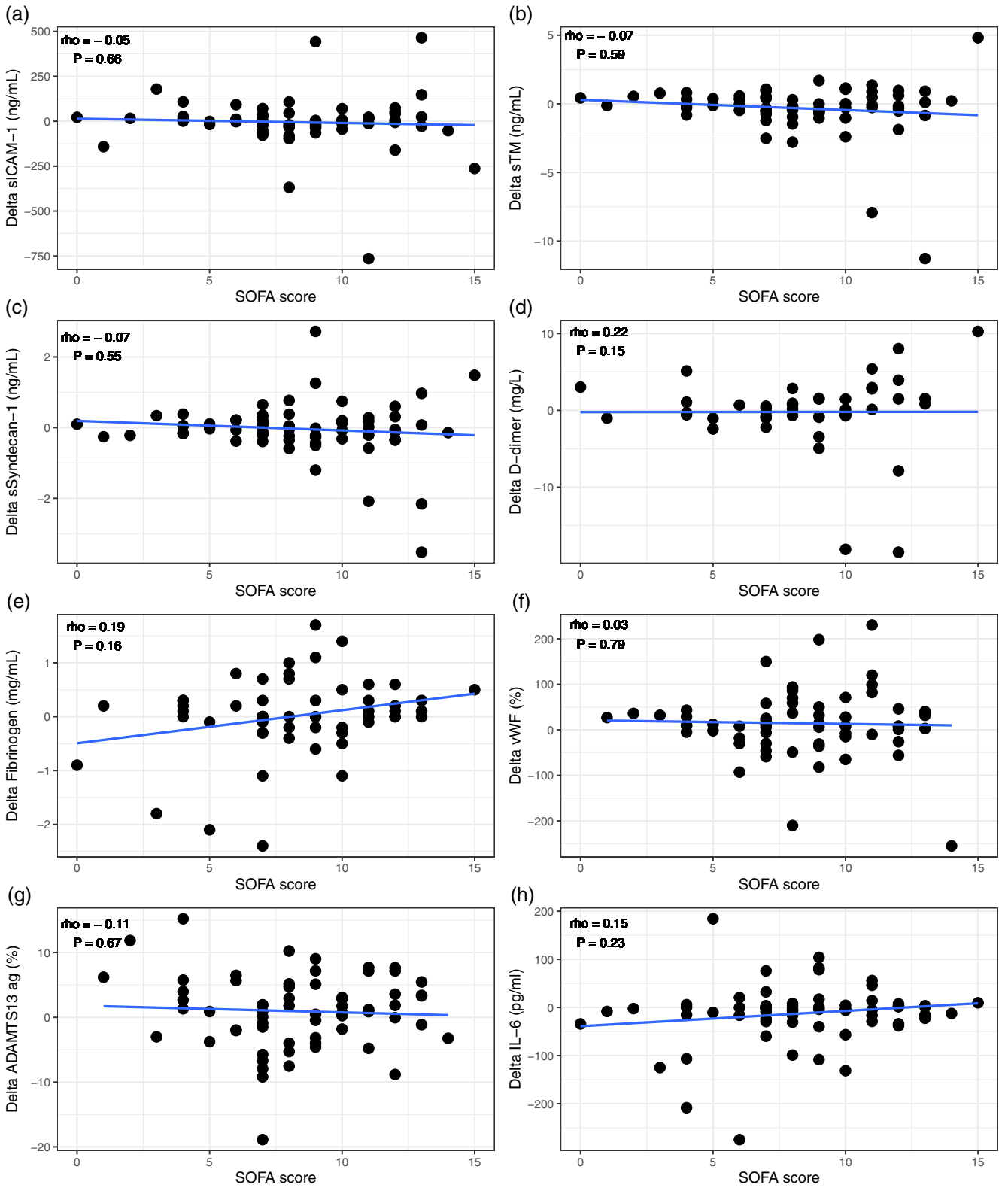


FIGURE 1 (a–h) correlation plots between post transfusion change in biomarker level and sequential organ failure assessment (SOFA) score. Rho = spearman rank correlation. TM = thrombomodulin. vWF = von Willebrand factor

worse outcomes [29], therefore more knowledge on the mechanism behind the association between RBC transfusion and thrombosis can be of great importance. By unravelling the mechanism responsible for transfusion related adverse events we may identify the responsible compounds in the RBC unit [30], enabling improvement of the transfusion product or protocols.

We did not observe an increase in biomarkers of endothelial cell damage such as syndecan-1 (a marker of glycocalyx degradation) and thrombomodulin (a glycoprotein on the endothelial cell membrane released into the circulation by endothelial damage), suggesting that RBC transfusion leads to activation but not to endothelial cell damage. However, soluble ICAM-1, which is also a marker of endothelial cell activation, did not increase after transfusion. A possible explanation is the high baseline level of soluble ICAM-1 before transfusion in the critically ill patient. The expression of ICAM-1 on the cell surface may already be saturated and therefore a further increase in the shedding of soluble ICAM-1 after transfusion was not possible. Furthermore, in preterm neonates, an increase in sICAM-1 was seen after the third RBC transfusion and not after the first or second [31]. Again, the impact of one RBC unit may have been too small to cause an increase in sICAM-1 in this study.

Sepsis is a risk factor for the development of TRALI [5], however, in this study, sepsis did not impact the effect of RBC transfusion on the measured biomarkers. Possibly, biomarkers were already too much deranged due to the critical illness before transfusion.

This study has several limitations. First, this study was not powered to correlate the increase of vWF antigen levels to transfusion-related adverse event, such as thrombo-embolic events. Also, as ICU patients undergo multiple interventions during their stay at the ICU, it is possible that the increase in vWF occurs in all ICU patients, regardless of the transfusion of RBCs. We did not include a control group to exclude this possibility. However, as vWF levels did not further increase at 48 h, it appears unlikely that this observed increase reflects a natural trajectory in these patients.

In conclusion, RBC transfusion in critically ill patients is associated with an increase of circulating vWF antigen levels, suggesting a further increase in activation of the endothelium. This finding was independent of the presence of sepsis or organ injury level. The clinical impact of the increase of vWF cannot be distracted from this study. Our findings highlight the importance for future research to focus on the association between multiple RBC transfusions, mediators of endothelial activation such as vWF increase, and the occurrence of adverse events.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Lisa van Manen https://orcid.org/0000-0001-6777-7875

REFERENCES

1. Vincent JL, Jaschinski U, Wittebole X, Lefrant JY, Jakob SM, Almekhlafi GA, et al. Worldwide audit of blood transfusion practice in critically ill patients. Crit Care. 2018;22:102. https://doi.org/10.1186/s13054-018-2018-9.
2. Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. Crit Care Med. 2008;36:2667–74.
3. Silvain J, Pena A, Cayla G, Brieger D, Bellemain-Appaix A, Chastre T, et al. Impact of red blood cell transfusion on platelet activation and aggregation in healthy volunteers: results of the TRANSFUSION study. Eur Heart J. 2010;31:2816–21.
4. Ghazi L, Schwann TA, Engoren MC, Habib RH. Role of blood transfusion product type and amount in deep vein thrombosis after cardiac surgery. Thromb Res. 2015;136:1204–10.
5. Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. Lancet. 2013;382:984–94.
6. Johansson PI, Stensballe J, Ostrowski SR. Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. Crit Care. 2017;21:25.
7. Hod EA, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, et al. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood. 2010;115:4284–92.
8. Vlaar APJ, Hofstra JJ, Levi M, Kulik W, Nieuwland R, Tool ATJ, et al. Supernatant of aged erythrocytes causes lung inflammation and coagulopathy in a "two-hit" in vivo syngeneic transfusion model. Anesthesiology. 2010;113:92–103.
9. Straat M, Tuip A, Klei TRL, Beuger BM, Roelofs JJTH, van Bruggen R, et al. Endotoxemia results in trapping of transfused red blood cells in lungs with associated lung injury. Shock. 2017;48:484–9.
10. Hod EA, Brittenham GM, Billote GB, Francis RO, Ginzburg YZ, Hendrickson JE, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. Blood. 2011;118:6675–82.
11. Keir AK, McPhee AJ, Andersen CC, Stark MJ. Plasma cytokines and markers of endothelial activation increase after packed red blood cell transfusion in the preterm infant. Pediatr Res. 2013;73:75–9.
12. Larsen AM, Leinøe EB, Johansson PI, Birgens H, Ostrowski SR. Haemostatic function and biomarkers of endothelial damage before and after RBC transfusion in patients with haematologic disease. Vox Sang. 2015;109:52–61.
13. Vlaar APJ, Cornet AD, Hofstra JJ, Porcelijn L, Beishuizen A, Kulik W, et al. The effect of blood transfusion on pulmonary permeability in cardiac surgery patients: a prospective multicenter cohort study. Transfusion. 2012;52:82–90.
14. Stock C, Riethmuller C. Endothelial activation drives lateral migration and diapedesis of leukocytes. Cell Immunol. 2011;271:180–3.
15. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med. 2008;359:938–49.
16. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315:801. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)–10.
17. Roose E, Tersteeg C, Demeersseman R, Schelpe A-S, Deforche L, Pareyn I, et al. Anti-ADAMTS13 antibodies and a novel heterozygous p.R1177Q mutation in a case of pregnancy-onset immune-mediated thrombotic thrombocytopenic purpura. TH Open. 2018;2:e8–15.
18. Roose E, Schelpe AS, Joly BS, Peetermans M, Verhamme P, Voorberg J, et al. An open conformation of ADAMTS-13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura. J Thromb Haemost. 2018;16:378–88.
19. Dekimpe C, Roose E, Tersteeg C, Joly BS, Dewaele A, Horta S, et al. Anti-ADAMTS13 autoantibodies in immune-mediated thrombotic thrombocytopenic purpura do not hamper ELISA-based quantification of ADAMTS13 antigen. J Thromb Haemost. 2020;18:985–90.
20. Feys HB, Roodt J, Vandeputte N, Pareyn I, Lamprecht S, van Rensburg WJ, et al. Thrombotic thrombocytopenic purpura directly linked with ADAMTS13 inhibition in the baboon (Papio ursinus). Blood. 2010;116:2005–10.
21. Deforche L, Roose E, Vandenbulcke A, Vandeputte N, Feys HB, Springer TA, et al. Linker regions and flexibility around the

metalloprotease domain account for conformational activation of ADAMTS-13. J Thromb Haemost. 2015;13:2063–75.
22. Plautz WE, Matthay ZA, Rollins-Raval MA, Rollins-Raval MA, Raval JS, Kornblith LZ, et al. Von Willebrand factor as a thrombotic and inflammatory mediator in critical illness. Transfusion. 2020;60: S158–66.
23. Silvain J, Abtan J, Kerneis M, Martin R, Finzi J, Vignalou JB, et al. Impact of red blood cell transfusion on platelet aggregation and inflammatory response in anemic coronary and noncoronary patients. J Am Coll Cardiol. 2014;63:1289–96.
24. Reitsma PH, Branger J, van den Blink B, Weijer S, van der Poll T, Meijers JCM. Procoagulant protein levels are differentially increased during human endotoxemia. J Thromb Haemost. 2003;1:1019–23.
25. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Tracy RP, Aleksic N, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). Am J Med. 2002;113:636–42.
26. Ocak G, Vossen CY, Verduijn M, Dekker FW, Rosendaal FR, Cannegieter SC, et al. Risk of venous thrombosis in patients with major illnesses: results from the MEGA study. J Thromb Haemost. 2013;11:116–23.
27. Goel R, Patel EU, Cushing MM, Frank SM, Ness PM, Takemoto CM, et al. Association of perioperative red blood cell transfusions with Venous Thromboembolism in a north American registry. JAMA Surg. 2018;153:826–33.
28. Russell L, Holst LB, Lange T, Liang X, Ostrowski SR, Perner A. Effects of anemia and blood transfusion on clot formation and platelet function in patients with septic shock: a substudy of the randomized TRISS trial. Transfusion. 2018;58:2807–18.
29. Malato A, Dentali F, Siragusa S, Fabbiano F, Kagoma Y, Boddi M, et al. The impact of deep vein thrombosis in critically ill patients: a meta-analysis of major clinical outcomes. Blood Transfus. 2015;13: 559–68.
30. Remy KE, Hall MW, Cholette J, Juffermans NP, Nicol K, Doctor A, et al. Mechanisms of red blood cell transfusion-related immunomodulation. Transfusion. 2018;58:804–15.
31. Crawford TM, Andersen CC, Stark MJ. Effect of repeat transfusion exposure on plasma cytokine and markers of endothelial activation in the extremely preterm neonate. Transfusion. 2020; 60:2217–24.

How to cite this article: van Manen L, van Hezel ME, Boshuizen M, Straat M, de Man AME, Dekimpe C, et al. Effect of red blood cell transfusion on inflammation, endothelial cell activation and coagulation in the critically ill. Vox Sang. 2022; 117:64–70.

Received: 4 January 2021 | Revised: 12 April 2021 | Accepted: 15 April 2021

DOI: 10.1111/vox.13128

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Investigation of patient factors associated with the number of transfusions required during chemotherapy for high-risk neuroblastoma

Saori Konno[1,2,3] | Ryu Yanagisawa[1,2,4] | Noriko Kubota[5] | Yoshifumi Ogiso[5] | Noriyuki Nishimura[6] | Kazuo Sakashita[7] | Minoru Tozuka[1,5]

[1]Life Science Research Centre, Nagano Children's Hospital, Azumino, Japan

[2]Division of Blood Transfusion, Shinshu University Hospital, Matsumoto, Japan

[3]Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan

[4]Centre for Advanced Cell Therapy, Shinshu University Hospital, Matsumoto, Japan

[5]Department of Laboratory Medicine, Nagano Children's Hospital, Azumino, Japan

[6]Department of Public Health, Kobe University Graduate School of Health Science, Kobe, Japan

[7]Department of Haematology and Oncology, Nagano Children's Hospital, Azumino, Japan

Correspondence
Ryu Yanagisawa, Division of Blood Transfusion, Shinshu University Hospital, 3-1-1, Asahi, Matsumoto 390-8621, Japan.
Email: ryu@shinshu-u.ac.jp

Abstract

Background: Blood transfusion is an important supportive care for high-risk neuroblastoma. When the number of transfusions increases, transfusion-associated adverse reactions may be more problematic. However, the factors determining the degree of myelosuppression and the number of transfusions during chemotherapy for high-risk neuroblastoma remain unclear.

Materials and Methods: We investigated patient factors determining the number of required transfusions in 15 high-risk neuroblastoma patients who received five courses of chemotherapy. Clinical data, cytokine profile and colony-forming assay with bone marrow samples at diagnosis were analysed.

Results: The required number of transfusions of both platelets and erythrocytes decreased once in the second course and then increased as the course progressed. The variability among cases increased as the chemotherapy course progressed. In cases of low peripheral blood platelet count and lower fibrinogen level at diagnosis, the number of platelet transfusions was higher during chemotherapy. In contrast, there was a negative correlation between the forming ability of granulocyte–macrophage or erythroid colonies and the number of erythrocyte transfusions in the latter period.

Conclusion: In the early stages of chemotherapy, bone marrow infiltration in neuroblastoma and/or coagulopathy complication may cause thrombocytopenia and requirement of platelet transfusion; conversely, in the later stages, the number of erythrocyte transfusions may be defined by the patient's inherent hematopoietic ability. These factors may be useful in predicting the required number of transfusions.

KEYWORDS
anaemia, BFU-E, clonal cell culture, colony-forming assay, thrombocytopenia

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumour of childhood characterized by undifferentiated neuroectodermal cells derived from the neural crest [1,2]. While some neuroblastomas occurring in infants younger than 18 months spontaneously regress, high-risk neuroblastomas continue to result in poor prognosis even when treated with various chemotherapeutic agents and

hematopoietic stem cell transplants after high-dose chemotherapy [3,4]. Chemotherapy for high-risk neuroblastoma usually causes severe myelosuppression due to repeated intensive regimens [4,5]. Use of erythropoietin and granulocyte-colony-stimulating factor does not reduce the number of transfusions in high-risk neuroblastoma chemotherapy [6]. Furthermore, use of erythropoietin may be not recommended for high-risk neuroblastoma chemotherapy patients because it is suggested that erythropoietin might support neuroblastoma metastatic cells [7]. Currently, multiple blood transfusion is an indispensable supportive care provided during chemotherapy [8]. However, the factors determining the degree of myelosuppression and the number of blood transfusions required during chemotherapy for high-risk neuroblastoma remain unclear.

On the contrary, increasing the number of transfusions may also elevate the risk of transfusion-associated adverse reactions and post-transfusion infections [9]. Despite the fact that treatment for the underlying disease is complete and long-term survival can be achieved, some long-term side-effects such as iron overload also occur [10]. Moreover, immunological modification by blood transfusion may have an effect on prognosis even in neuroblastoma [11]. Furthermore, if the patient has a blood type with a low donor frequency, such as Rh-negative red blood cell (RBC) type, then it may be difficult to secure the necessary blood products. Therefore, if the factors related to anaemia and thrombocytopenia caused by chemotherapy are clarified and the required number of blood transfusions in each case can be predicted to some extent, then it will be easier to take measures for these problems. Additionally, prediction of the required number of blood transfusions in each case will lead to proper use of limited blood products.

The results of previous studies conducted in adults reveal that both chemotherapy factors (e.g. anticancer agent or regimen used, duration of chemotherapy) and patient factors (e.g. disease status, patient's condition and patient's laboratory data) determine the required number of blood transfusions [12–21]. Chemotherapy factors include information that can be shared with other diseases for which a similar regimen is used. However, clarifying patient factors may contribute to the elucidation of the pathophysiology of the disease. This information may also be useful in the clinical practice of personalized medicine for each patient. We therefore aimed to determine the number of blood transfusions required during almost similar chemotherapy for high-risk neuroblastoma and clarified patient factors, particularly those related to the number of blood transfusions required.

MATERIALS AND METHODS

Patient's selection and collection of patient's clinical information

Between January 2002 and December 2016, 15 neuroblastoma patients who met the following conditions and who were diagnosed and treated in the Department of Haematology and Oncology, Nagano Children's Hospital, were enrolled. Patients were diagnosed with Stage 3 or Stage 4 neuroblastoma using the International Neuroblastoma Staging System (INSS) and received a total of five courses of chemotherapy as proposed by the Japan Neuroblastoma Study Group [3,4]. Patients underwent treatment for high-risk neuroblastoma, which was defined by Children's Oncology Group Criteria [22]. The JANB98 protocol was used for treatment during 2002–2007, and the JN-H-07 protocol was used for treatment during 2008–2016 in the institution [3,4]. The number of RBC and platelet (PLT) transfusions required during 1–5 chemotherapy courses was investigated in all cases. In addition to clinical information collected at the time of diagnosis, data on haematological values at the start of each chemotherapy course were analysed as patient factors related to the required number of blood transfusions. As for the analysis of bone marrow metastasis, the presence or absence of tyrosine hydroxylase was evaluated using RT-PCR apart from the pathological visual findings [23]. Cases in which tyrosine hydroxylase was not measured at the time of diagnosis were analysed separately using preserved bone marrow cells [24,25]. This study was approved by the Nagano Children's Hospital Institutional Review Board (approval number: 30-38, 30-54 and 31-66).

Blood transfusion

Red blood cell or PLT transfusion was performed using a haemoglobin level of 70 g/L and a PLT level of 20×10^9/L as criteria for trigger values during the study period. In Japan, guidelines for PLT transfusion were published in 2017 as a trigger value of 10×10^9/L for chemotherapy for cancer diseases [26,27]. All of the cases in this study were analysed before the publication of these guidelines, and prophylactic PLT transfusion was performed according to standards different from those followed in other countries [28]. Details of the blood products transfused are described in previous papers [29].

Colony-forming assay

Colony-forming assay, using stored bone marrow mononucleosis collected at initial diagnosis, was performed by the technique described previously [30,31]. CD34+ cells were selected and enriched from bone marrow mononuclear cells by positive immunomagnetic selection with the CD34 MicroBeads Kit, human (Miltenyi Biotec, Bergisch Gladbach, Germany) [30]. Cell cultures were prepared in 35-mm Lux suspension culture dishes (#153066; Nunc, Roskilde, Denmark). Twelve hundred CD34+ cells were divided into four dishes containing Methocult™ H4435 Enriched (Stemcell Technologies, Vancouver, BC, Canada). The dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO_2. On day 14, the total number of granulocyte-macrophage (GM) colonies (colony-forming unit-granulocyte/macrophage [CFU-GM], colony-forming unit-granulocyte [CFU-G] and colony-forming unit-macrophage [CFU-M]) and erythroid colonies (burst-forming unit-erythroid [BFU-E]) were scored as the average value per dish in situ under an inverted microscope. We also analysed megakaryocytic progenitor cells using the MegaCult™-C Complete Kit with Cytokines (Stemcell Technologies, Vancouver, BC, Canada). Briefly, CD34+ cells were added at a density of $2{\cdot}5 \times 10^3$

cells per chamber (in 0·75 ml of medium) of a double-chamber slide containing Megacult^{TM}-C medium with cytokines. After incubation at 37°C with 5% CO_2 for 12 days, the samples were dehydrated and fixed, and total colony-forming unit-megakaryocytes (CFU-MK) were stained with monoclonal anti-CD41 (GPIIb/IIIa). Because of the limitation of stored bone marrow samples, GM colonies and BFU-E were analysed in a total of 10 cases, and CFU-MK was analysed in 7 cases.

Analysis of the cytokine profile

In this study, we planned to analyse several cytokines that are thought to be closely involved in the hematopoiesis of erythrocytes and PLT [32–35]. The cytokines present in peripheral blood at the initial diagnosis of neuroblastoma were analysed as follows: Interleukin (IL)-1β, IL-3, IL-6, IL-10, IL-11, IL-13, GM colony-stimulating factor, stem cell factor, leukaemia inhibitory factor, thrombopoietin, interferon (IFN)-γ, tumour necrosis factor (TNF)-α and fibroblast growth factors (FGF) were analysed using the Human Premixed Multi-Analyte Kit (R&D systems, Minneapolis, MN, USA). Transforming growth factor (TGF)-β1 was analysed using the Human TGF-beta 1 Quantikine ELISA Kit (R&D Systems). All of these cytokines were measured using the Enzyme-Linked Immuno Sorbent Assay. Erythropoietin was analysed using Access EPO (Beckman Coulter, CA, USA) by chemiluminescent enzyme immunoassay. The cytokine profile was analysed in 11 cases, with the available samples.

Statistical analysis

The Friedman test was used to compare the required number of blood transfusions between each chemotherapy course and the haematological values (white blood cell [WBC] count, PLT count, haemoglobin level and reticulocyte count) at the start of each chemotherapy course. The difference in the required number of blood transfusions depending on the patient background characteristics or clinical data at diagnosis was examined using the Mann–Whitney U-test. The correlation between the required number of blood transfusions and the results of clinical data or colony-forming assay and the correlation between the cytokine profile and the results of clinical data or colony-forming assay were analysed using Spearman's rank correlation coefficient. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [36]. Statistical significance was defined as P <0·050.

RESULTS

Haematological values at the start of each chemotherapy course and the required number of blood transfusions

Figure 1 shows the changes in WBC count, PLT count, haemoglobin level and reticulocyte count at the initiation of chemotherapy during chemotherapy courses 1–5. The WBC count tended to decrease significantly as the course progressed, indicating gradual progression of myelosuppression. However, the PLT count increased at the start of the second chemotherapy course compared to that at the start of the first course, which then gradually decreased. There was no significant difference in the change between haemoglobin level and reticulocyte count throughout the chemotherapy course.

Figure 2 shows the number of RBC transfusions and PLT transfusions performed in each of the first to fifth chemotherapy courses. Both RBC and PLT blood transfusions decreased in the second course compared to that in the first course, which then increased as the course progressed. The number of blood transfusions tended to vary with each case as the chemotherapy course progressed.

Required number of blood transfusions due to differences in patient background characteristics at diagnosis

Table S1 shows the differences in the required number of RBC transfusions, number of PLT transfusions and total number of blood transfusions depending on the patient background characteristics in this study.

Among all of the evaluated parameters, cases with a low PLT count at diagnosis had a high frequency of RBC transfusion, PLT transfusion and the total number of blood transfusion. Furthermore, in cases where the fibrinogen level was low at diagnosis, the number of PLT transfusions and the total number of transfusions were higher. Data could not be collected for all cases; however, the total number of transfusions was also higher in the D-dimer high-value cases. Although the same drug was used in the JANB98 and JN-H-07 protocols, the total dose of cisplatin differed depending on the case; however, the number of required blood transfusions did not differ. Although no significant difference was observed, the group with bone marrow metastasis tended to receive more PLT transfusions ($P = 0·059$) and total number of blood transfusions ($P = 0·069$) than the non-metastasis group.

Next, according to these results, as shown in Table S1, we analysed the correlation between PLT count or fibrinogen level at diagnosis and the number of RBC transfusions and PLT transfusions performed in each chemotherapy course (Tables 1 and 2). All results showed a negative tendency but a significant negative correlation, especially the number of required PLT transfusions. The required number of RBC transfusions in some chemotherapy courses also showed a negative correlation with the PLT count and fibrinogen levels at diagnosis.

Results of colony-forming assay with bone marrow cells at diagnosis and the required number of blood transfusions

Table 3 shows the relationship between the required number of RBCs and PLT transfusions and the results of the colony-forming assay using bone marrow cells at the time of diagnosis. Both the number of

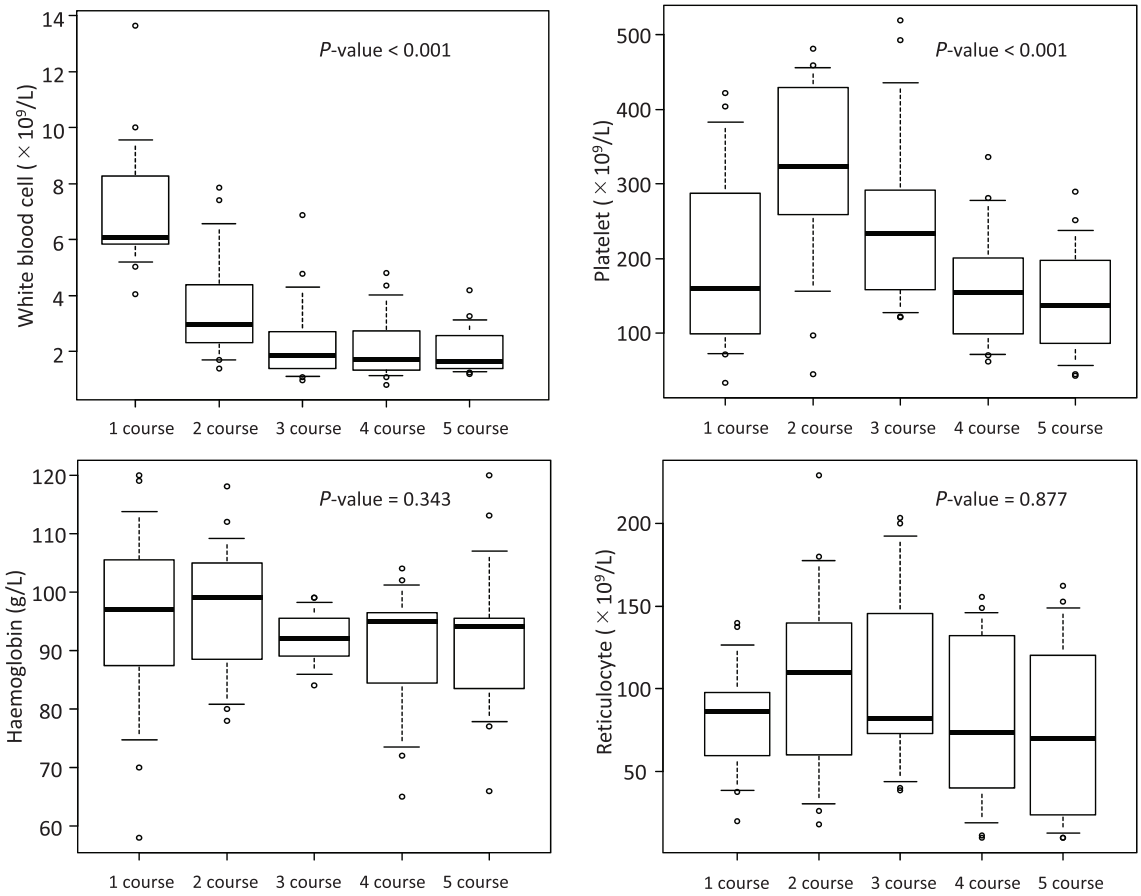


FIGURE 1 Changes in the haematological value of peripheral blood at the beginning of the first to the fifth chemotherapy course

GM colonies and BFU-E were negatively correlated with the required number of RBC transfusions in the third, fourth and fifth courses of chemotherapy and analysis of total chemotherapy courses. There was also a negative correlation between the number of GM colonies/BFU-E and the required number of PLT transfusions only during the second course of chemotherapy. However, the results of the CFU-MK number did not show any significant correlation with the required number of RBC or PLT transfusions.

Cytokine profile at diagnosis and PLT count, fibrinogen level and colony-forming assay

From the results of these studies, the peripheral blood PLT count and the fibrinogen level at the time of diagnosis showed a negative correlation, especially with the required number of PLT transfusions. In addition, the ability of bone marrow to form GM colonies or BFU-E before treatment showed a negative correlation particularly with the number of required RBC transfusions.

Focusing on these parameters, we analysed the relationship between cytokines that may be associated with PLT and erythroid hematopoiesis and PLT count, fibrinogen level and colony-forming assay. The results revealed that the PLT count before chemotherapy showed a negative correlation with IL-3 but a positive correlation with TGF-β1. In contrast, GM colonies and BFU-E-forming ability in bone marrow cells before chemotherapy were positively correlated with both IL-13 and TNF-α. However, there was no correlation with any cytokine in terms of fibrinogen levels (Table 4).

DISCUSSION

There is little evidence regarding how the haematological value before each chemotherapy course changes and how the number of transfusions required for each chemotherapy course changes in high-risk neuroblastoma. In this study, the WBC count was highest at the start of the first course, and it was shown that myelosuppression progressed steadily with repeated chemotherapy. However, the PLT count increased significantly at the start of the second course compared to that the start of the first course. In addition, the frequency of RBC and PLT transfusions during the second course of chemotherapy was the lowest. Then, the required number of blood transfusions for RBCs and PLTs was characterized by increasing individual differences as the chemotherapy course progressed. Furthermore, it was shown that the peripheral blood PLT count and the fibrinogen level at the time of diagnosis and the GM colonies or BFU-E-forming ability of initial bone marrow cells may be useful indicators of the required number of PLT or RBC blood transfusion.

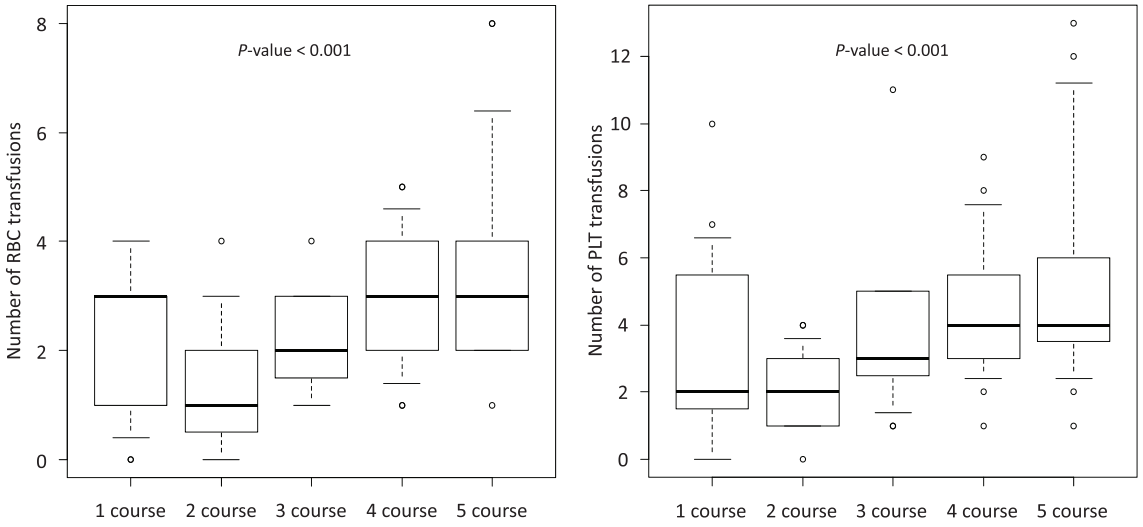


FIGURE 2 Number of required blood transfusions during each chemotherapy course. RBC, red blood cell; PLT platelet

TABLE 1 Relationship between peripheral blood platelet count at diagnosis and the required number of blood transfusions

(A) RBC transfusion		
Course of chemotherapy	***r***	***p*-value**
1	-0.521	**0.047**
2	-0.448	0.094
3	-0.517	**0.048**
4	-0.463	0.083
5	-0.301	0.276
Total	-0.691	**0.004**
(B) PLT transfusion		
Course of chemotherapy	***r***	***p*-value**
1	-0.804	**<0.001**
2	-0.610	**0.016**
3	-0.753	**0.001**
4	-0.511	0.052
5	-0.627	**0.012**
Total	-0.837	**<0.001**

Note: Bold type: statistical significance ($P < 0.050$).
Abbreviations: PLT, platelet; RBC, red blood cell.

TABLE 2 Relationship between blood fibrinogen level at diagnosis and the required number of blood transfusions

(A) RBC transfusion		
Course of chemotherapy	***r***	***p*-value**
1	-0.288	0.297
2	-0.177	0.528
3	-0.564	**0.029**
4	-0.304	0.271
5	-0.315	0.252
Total	-0.503	0.056
(B) PLT transfusion		
Course of chemotherapy	***r***	***p*-value**
1	-0.669	**0.006**
2	-0.575	**0.025**
3	-0.591	**0.020**
4	-0.518	**0.048**
5	-0.618	**0.014**
Total	-0.736	**0.002**

Note: Bold type: statistical significance ($P < 0.050$).
Abbreviations: PLT, platelet; RBC, red blood cell.

In a previous study, the following variables other than chemotherapy factors have been reported as patient factors related to the number of prophylactic PLT transfusions required during chemotherapy in adults; low PLT count, low lymphocyte count, low fibrinogen, low mean PLT volume, high PLT aggregation, high albumin level and greater PS score [19,20]. Although all of these variables could not be evaluated in our study, the lower PLT count and the lower fibrinogen level were common risk factors in our analyses. One mechanism of thrombocytopenia with neuroblastoma at diagnosis is thought to be as follows: the proliferation of neuroblastoma cells in the bone marrow reduces normal hematopoiesis, [37] neuroblastoma cells may also have a direct inhibitory effect on bone marrow cells [38]. Therefore, thrombocytopenia is thought to be a sensitive reflection of the effects of metastasis of neuroblastoma cells, although erythrocytes have a long life span and anaemia may not be immediately pronounced without such as intratumoral haemorrhage. Contrarily, coagulopathy complications could also occur in advanced neuroblastoma, which shows a decrease in PLT count, fibrinogen and other coagulation factors, especially when accompanied by disseminated intravascular clotting [39–41]. Although it is considered that the first

TABLE 3 Relationship between the results of colony forming assay using bone marrow cells at diagnosis and the required number of blood transfusions

(A) RBC transfusion						
Course of chemotherapy	GM colonies		BFU-E		CFU-MK	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
1	0.465	0.175	0.379	0.279	-0.295	0.520
2	-0.604	0.065	-0.591	0.072	0.373	0.410
3	-0.671	**0.034**	-0.641	**0.046**	0.659	0.107
4	-0.873	**0.001**	-0.893	**0.001**	0.588	0.165
5	-0.769	**0.009**	-0.788	**0.007**	0.335	0.463
Total	-0.669	**0.035**	-0.704	**0.023**	0.395	0.381
(B) PLT transfusion						
Course of chemotherapy	GM colonies		BFU-E		CFU-MK	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
1	0.031	0.933	0.050	0.891	-0.370	0.413
2	-0.649	**0.042**	-0.646	**0.044**	0.558	0.193
3	-0.329	0.353	-0.470	0.171	-0.113	0.809
4	-0.429	0.216	-0.524	0.120	0.111	0.813
5	-0.247	0.492	-0.191	0.597	-0.120	0.797
Total	-0.280	0.432	-0.384	0.273	-0.306	0.505

Note: Bold type: statistical significance ($P < 0.050$).
Abbreviations: BFU-E, burst-forming unit-erythroid; CFU-MK, colony-forming unit-megakaryocyte; GM colonies, colony forming unit-granulocyte/macrophage, colony-forming unit-granulocyte, and colony-forming unit-macrophage; PLT, platelet; RBC, red blood cell.

TABLE 4 Relationship between the cytokine prof ile of peripheral blood at diagnosis, platelet count, f ibrinogen level, and results of colony-forming assay

	PLT count		Fibrinogen level		GM colonies		BFU-E	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
IL-1β	-0.159	0.640	-0.036	0.915	0.335	0.417	0.061	0.887
IL-3	-0.703	**0.016**	-0.298	0.374	0.610	0.108	0.383	0.349
IL-6	0.427	0.193	0.409	0.214	-0.119	0.793	-0.253	0.545
IL-10	-0.310	0.354	-0.451	0.164	0.571	0.151	0.687	0.060
IL-11	-0.584	0.059	-0.468	0.146	0.592	0.122	0.418	0.303
IL-13	-0.378	0.252	-0.296	0.377	0.762	**0.037**	0.735	**0.038**
GM-CSF	-0.116	0.735	-0.021	0.951	0.592	0.122	0.418	0.303
SCF	-0.255	0.451	-0.464	0.154	0.095	0.840	-0.024	0.955
LIF	-0.584	0.059	-0.468	0.146	0.592	0.122	0.418	0.303
EPO	0.209	0.539	0.018	0.968	-0.500	0.216	-0.265	0.526
TPO	-0.455	0.163	-0.455	0.163	0.262	0.536	0.193	0.647
IFN-γ	-0.536	0.094	-0.491	0.129	0.405	0.327	0.349	0.396
TNF-α	-0.527	0.100	-0.036	0.924	0.810	**0.022**	0.831	**0.011**
TGF-β1	0.767	**0.021**	0.283	0.463	-0.107	0.840	-0.055	0.908
FGF	-0.200	0.558	-0.218	0.521	0.429	0.299	0.361	0.379

Note: Bold type: statistical significance ($P < 0.050$).
Abbreviations: BFU-E, burst-forming unit-erythroid; EPO, erythropoietin; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; GM colonies, colony-forming unit-granulocyte/macrophage, colony-forming unit-granulocyte, and colony-forming unit-macrophage; IFN, Interferon; L, interleukin; LIF, leukaemia inhibitory factor; PLT, platelet; RBC, red blood cell; SCF, stem cell factor; TGF, transforming growth factor; TNF, tumour necrosis factor; TPO, thrombopoietin.

chemotherapy reduced the number of neuroblastoma cells in the bone marrow and restored the PLT-producing ability, and PLT consumption is improved by normalization of coagulation abnormalities, dependence on PLT transfusions may continue to be affected during subsequent chemotherapies for neuroblastoma.

In analysing the cytokine profile in peripheral blood at diagnosis, the number of peripheral blood PLTs showed a positive correlation with TGF-β1 and a negative correlation with IL-3. IL-3 is known to multiply CFU-MK [42–44]. In addition, when IL-3 was administered to the human body, an increase in PLTs was actually observed, [45] and it is one of the typical cytokines that promote PLT production. Nevertheless, TGF-β1 acts in a suppressive manner on CFU-MK, megakaryocyte growth and endomitosis [46,47]. In addition, TGF-β1 is contained in the PLT alpha granule, and it is considered that when the number of PLTs increases, TGF-β1 in the blood also increases, and therefore, it has an effect of suppressing PLT production [46,48–50]. Therefore, the IL-3 and TGF-β1 profile results may reflect the body's response to compensate for the PLT-producing ability suppressed by neuroblastoma invasion into the bone marrow and/or PLT consumption by coagulopathy. Although the measurement of peripheral blood PLT count or fibrinogen level is a simple method for routine testing, it may show a good reflection of a disease background and may be a good sensitive predictor of the required number of PLT transfusions.

However, patient risk factors for anaemia requiring red blood cell (RBC) transfusion due to chemotherapy in the adult cancer area were older age, lower body mass index, greater performance status (PS), primary tumour site, tumour metastasis, prior blood transfusion, baseline low haemoglobin value, low haematocrit value, low lymphocyte count, high haptoglobin and high ferritin levels [15-18,21]. Although there are few studies on the chemotherapy regimen used for paediatric solid tumours including neuroblastoma, haemoglobin <12 g/dl, absolute lymphocyte count ≤700/μl and PS >1 before chemotherapy are reported as risk factors for severe anaemia requiring RBC transfusion [51]. However, theabovementioned parameters could not be found as risk factors in the present study, the result was related to the ability of bone marrow cells to form GM colonies and BFU-E at diagnosis. These results are considered to reflect the myelopoietic or erythropoietic ability originally possessed by each case. However, in the cytokine assay of peripheral blood, the ability of both formations showed a positive correlation with IL-13 and TNF-α. IL-13 and TNF-α are usually known to function in a suppressive manner in erythrocyte hematopoiesis [52–54]. Therefore, it is considered that the profiles of IL-13 and TNF-α in this study might be different from those that directly affect the ability to form GM colonies or BFU-E. Another possible reason for this result is that tumour infiltration by lymphocytes was reported to improve clinical outcome in children with neuroblastoma, [55–57] and tumour-associated T lymphocytes may play an important role in neuroblastoma [58]. Peripheral blood lymphocytes from neuroblastoma patients also produced relatively high levels of cytokines including IL-13 and TNF-α. Furthermore, the increased levels of these cytokines may be observed in co-cultures of patients' lymphocytes with tumour cells [55]. Therefore, IL-13 and TNF-α may reflect the results of cytokine production by the activation of T lymphocytes [59]. Furthermore, TNF-α has been shown to have an inhibitory effect on neuroblastoma in vitro [60,61]. Therefore, in cases where IL-13 and TNF-α are high, it is possible that they have a protective effect on bone marrow hematopoietic cells and have well-maintained hematopoietic ability from neuroblastoma cells. However, this is still in the speculated stage at this point, and further basic verification is required in the future.

Unfortunately, there are many research limitations in this research. One of the limitations of this study was the retrospective analysis of a small number of single-centre cases. Because this study targeted paediatric patients, the amount of residual sample was small and limited; therefore, colony-forming assay and cytokine profile could not be analysed in all cases. In addition, because the era in which chemotherapy was performed was long, there is a possibility that some bias was added due to differences in supportive care. Furthermore, because only a limited number of cases of high-risk neuroblastoma were targeted, it is not clear whether the results obtained in this study could be relevant to other childhood cancer diseases and adult cancers. To solve such problems, it is necessary to perform prospective analysis for a large number of cases in the future. In addition, it is necessary to conduct a basic analysis together.

Through this study, we were able to clarify the changes in peripheral blood haematological value and the required number of blood transfusions when multiple chemotherapy treatments were administered for high-risk neuroblastoma. In addition, more PLT transfusions were required during chemotherapy in the low PLT count group and the low fibrinogen level group before the start of treatment. Moreover, patients with low GM colonies or BFU-E formation in the bone marrow before the start of treatment required more RBC transfusion during chemotherapy. The decrease in the number of peripheral blood PLTs is considered to be the result of a negative effect on bone marrow cells due to bone marrow infiltration caused by neuroblastoma and increased PLT consumption associated with abnormal coagulation. Furthermore, the ability to form GM colonies and BFU-E is considered to reflect the original hematopoietic ability of each case, and a correlation was observed, especially at the later stages of chemotherapy. By using these parameters, it may be possible to predict the degree of myelosuppression during chemotherapy and individual differences in the required number of blood transfusions. However, as there are many research limitations, further verification is needed in the future.

ACKNOWLEDGEMENTS

We thank Wiley Editing Services (https://wileyeditingservices.com/en/) for English language editing.

Study design: R.Y., K.S., and M.T.; data acquisition: S.K., N.K., and N.N.; data interpretation: S.K., R.Y., Y.O., and N.N.; manuscript draft preparation: S.K. and R.Y.; manuscript revision and approval: S.K., R.Y., N.K., Y.O., N.N., K.S., and M.T.

FUNDING INFORMATION

None.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Ryu Yanagisawa https://orcid.org/0000-0003-4012-0958

REFERENCES

1. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. Lancet 2007;369:2106–20.
2. Schwab M, Westermann F, Hero B, Berthold F. Neuroblastoma: biology and molecular and chromosomal pathology. Lancet Oncol 2003;4:472–80.
3. Nakagawara A, Li Y, Izumi H, Muramori K, Inada H, Nishi M. Neuroblastoma. Jpn J Clin Oncol 2018;48:214–41.
4. Hishiki T, Matsumoto K, Ohira M, et al. Results of a phase II trial for high-risk neuroblastoma treatment protocol JN-H-07: a report from the Japan Childhood Cancer Group Neuroblastoma Committee (JNBSG). Int J Clin Oncol 2018;23:965–73.
5. Saito S, Yanagisawa R, Minami K, et al. Prophylactic piperacillin administration in pediatric patients with solid tumors following different intensities of chemotherapy. Pediatr Int 2020;62:158–68.
6. Wagner LM, Billups CA, Furman WL, Rao BN, Santana VM. Combined use of erythropoietin and granulocyte colony-stimulating facto Although data did not exist in all cases r does not decrease blood transfusion requirements during induction therapy for high-risk neuroblastoma: a randomized controlled trial. J Clin Oncol 2004;22:1886–93.
7. Poniewierska-Baran A, Rajewska-Majchrzak J, Ratajczak MZ. Erythropoietin enhances migration of human neuroblastoma cells: in vitro studies and potential therapeutic implications. J Cancer Stem Cell Res 2017;5:e1003.
8. Silverstein A, Reddy K, Smith V, Foster JH, Russell HV, Whittle SB. Blood product administration during high risk neuroblastoma therapy. Pediatr Hematol Oncol 2020;37:5–14.
9. Savage WJ. Transfusion reactions. Hematol Oncol Clin North Am 2016;30:619–34.
10. Schempp A, Lee J, Kearney S, Mulrooney DA, Smith AR. Iron overload in survivors of childhood cancer. J Pediatr Hematol Oncol 2016;38:27–31.
11. Blumberg N, Triulzi DJ, Heal JM. Transfusion-induced immunomodulation and its clinical consequences. Transfus Med Rev 1990;4:24–35.
12. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. J Natl Cancer Inst 1999;91:1616–34.
13. Kuter DJ. Managing thrombocytopenia associated with cancer chemotherapy. Oncology (Williston Park) 2015;29:282–94.
14. Weycker D, Hatfield M, Grossman A, et al. Risk and consequences of chemotherapy-induced thrombocytopenia in US clinical practice. BMC Cancer 2019;19:151.
15. Coiffier B, Guastalla JP, Pujade-Lauraine E, Bastit P. Predicting cancer-associated anaemia in patients receiving non-platinum chemotherapy: results of a retrospective survey. Eur J Cancer 2001;37:1617–23.
16. Ray-Coquard I, Le Cesne A, Rubio MT, et al. Risk model for severe anemia requiring red blood cell transfusion after cytotoxic conventional chemotherapy regimens. The Elypse 1 Study Group. J Clin Oncol 1999;17:2840–6.
17. Razzaghdoust A, Mofid B, Peyghambarlou P. Predictors of chemotherapy-induced severe anemia in cancer patients receiving chemotherapy. Support Care Cancer 2020;28:155–61.
18. Okamoto H, Saijo N, Shinkai T, et al. Chemotherapy-induced anemia in patients with primary lung cancer. Ann Oncol 1992;3:819–24.
19. Blay JY, Le Cesne A, Mermet C, et al. A risk model for thrombocytopenia requiring platelet transfusion after cytotoxic chemotherapy. Blood 1998;92:405–10.
20. Vinholt PJ, Alnor A, Nybo M, Hvas A-M. Prediction of bleeding and prophylactic platelet transfusions in cancer patients with thrombocytopenia. Platelets 2016;27:547–54.
21. Heddens D, Alberts DS, Hannigan EV, et al. Prediction of the need for red cell transfusion in newly diagnosed ovarian cancer patients undergoing platinum-based treatment. Gynecol Oncol 2002;86:239–43.
22. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. Pediatr Clin North Am 2015;62:225–56.
23. Miyajima Y, Kato K, Numata S, et al. Detection of neuroblastoma cells in bone marrow and peripheral blood at diagnosis by the reverse transcriptase-polymerase chain reaction for tyrosine hydroxylase mRNA. Cancer 1995;75:2757–61.
24. Hirase S, Saitoh A, Hartomo TB, et al. Early detection of tumor relapse/regrowth by consecutive minimal residual disease monitoring in high-risk neuroblastoma patients. Oncol Lett 2016;12:1119–23.
25. Hartomo TB, Kozaki A, Hasegawa D, et al. Minimal residual disease monitoring in neuroblastoma patients based on the expression of a set of real-time RT-PCR markers in tumor-initiating cells. Oncol Rep 2013;29:1629–36.
26. Takami A, Matsushita T, Ogata M, et al. Guideline for the use of platelet transfusion concentrates based on scientific evidence. Jpn J Transfus Cell Ther 2017;63:569–84.
27. Takami A, Matsushita T, Ogata M, et al. Guideline for the use of platelet transfusion concentrates based on scientific evidence: update 2019. Jpn J Transfus Cell Ther 2019;65:544–61.
28. Schiffer CA, Bohlke K, Delaney M, et al. Platelet transfusion for patients with cancer: american society of clinical oncology clinical practice guideline update. J Clin Oncol 2018;36:283–99.
29. Yanagisawa R, Tatsuzawa Y, Ono T, et al. Analysis of clinical presentations of allergic transfusion reactions and febrile non-haemolytic transfusion reactions in paediatric patients. Vox Sang 2019;114:826–34.
30. Sakashita K, Koike K, Kinoshita T, et al. Dynamic DNA methylation change in the CpG island region of p15 during human myeloid development. J Clin Invest 2001;108:1195–204.
31. Sakashita K, Kato I, Daifu T, et al. In vitro expansion of CD34(+) CD38(-) cells under stimulation with hematopoietic growth factors on AGM-S3 cells in juvenile myelomonocytic leukemia. Leukemia 2015;29:606–14.
32. Deutsch VR, Tomer A. Advances in megakaryocytopoiesis and thrombopoiesis: from bench to bedside. Br J Haematol 2013;161:778–93.
33. Robb L. Cytokine receptors and hematopoietic differentiation. Oncogene 2007;26:6715–23.
34. Metcalf D. Hematopoietic cytokines. Blood 2008;111:485–91.
35. Nandakumar SK, Ulirsch JC, Sankaran VG. Advances in understanding erythropoiesis: evolving perspectives. Br J Haematol 2016;173:206–18.
36. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452–8.
37. Wang W, Peng X, Liang C, et al. Comparison of hematological aspects between neuroblastoma patients with and without bone marrow infiltration. Ann Clin Lab Sci 2017;47:416–21.
38. Sietsma H, Nijhof W, Dontje B, Vellenga E, Kamps WA, Kok JW. Inhibition of hemopoiesis in vitro by neuroblastoma-derived gangliosides. Cancer Res 1998;58:4840–4.
39. McMillan CW, Gaudry CL Jr, Holemans R. Coagulation defects and metastatic neuroblastoma. J Pediatr 1968;72:347–50.
40. Scott JP, Morgan E. Coagulopathy of disseminated neuroblastoma. J Pediatr 1983;103:219–22.

41. Hatae Y, Takeda T, Hattori T, Nakadate H, Nishi M. Advanced neuroblastoma and disseminated intravascular coagulation: report of six cases. Jpn J Clin Oncol 1985;15:483–8.
42. Dolzhanskiy A, Hirst J, Basch RS, Karpatkin S. Complementary and antagonistic effects of IL-3 in the early development of human megakaryocytes in culture. Br J Haematol 1998;100:415–26.
43. Lu L, Wang LS, Cooper RJ, et al. Suppressive effects of TNF-alpha, TGF-beta1, and chemokines on megakaryocytic colony formation in CD34+ cells derived from umbilical cord blood compared with mobilized peripheral blood and bone marrow. J Hematother Stem Cell Res 2000;9:195–204.
44. Teramura M, Katahira J, Hoshino S, et al. Clonal growth of human megakaryocyte progenitors in serum-free cultures: effect of recombinant human interleukin 3. Exp Hematol 1988;16:843–8.
45. Ganser A, Lindemann A, Seipelt G, et al. Effects of recombinant human interleukin-3 in patients with normal hematopoiesis and in patients with bone marrow failure. Blood 1990;76:666–76.
46. Mitjavila MT, Vinci G, Villeval JL, et al. Human platelet alpha granules contain a nonspecific inhibitor of megakaryocyte colony formation: its relationship to type beta transforming growth factor (TGF-beta). J Cell Physiol 1988;134:93–100.
47. Kuter DJ, Gminski DM, Rosenberg RD. Transforming growth factor beta inhibits megakaryocyte growth and endomitosis. Blood 1992; 79:619–26.
48. Kunz D, Luley C, Heim MU, Bock M. Transforming growth factor beta is increased in plasma of patients with hematologic malignancies after transfusion of platelet concentrates. Transfusion 1998;38:156–9.
49. Wakefield LM, Smith DM, Flanders KC, Sporn MB. Latent transforming growth factor-beta from human platelets. A high molecular weight complex containing precursor sequences. J Biol Chem 1988; 263:7646–54.
50. Fujihara M, Ikebuchi K, Wakamoto S, Sekiguchi S. Effects of filtration and gamma radiation on the accumulation of RANTES and transforming growth factor-beta1 in apheresis platelet concentrates during storage. Transfusion 1999;39:498–505.
51. Marec-Berard P, Blay JY, Schell M, et al. Risk model predictive of severe anemia requiring RBC transfusion after chemotherapy in pediatric solid tumor patients. J Clin Oncol 2003;21:4235–8.
52. Testa U. Apoptotic mechanisms in the control of erythropoiesis. Leukemia 2004;18:1176–99.
53. Cooper AC, Mikhail A, Lethbridge MW, et al. Increased expression of erythropoiesis inhibiting cytokines (IFN-gamma, TNF-alpha, IL-10, and IL-13) by T cells in patients exhibiting a poor response to erythropoietin therapy. J Am Soc Nephrol 2003;14: 1776–84.
54. Xi X, Schlegel N, Caen JP, et al. Differential effects of recombinant human interleukin-13 on the in vitro growth of human haemopoietic progenitor cells. Br J Haematol 1995;90:921–7.
55. Carlson LM, De Geer A, Sveinbjornsson B, et al. The microenvironment of human neuroblastoma supports the activation of tumor-associated T lymphocytes. Oncoimmunology 2013;2:e23618.
56. Martin RF, Beckwith JB. Lymphoid infiltrates in neuroblastomas: their occurrence and prognostic significance. J Pediatr Surg 1968;3: 161–4.
57. Lauder I, Aherne W. The significance of lymphocytic infiltration in neuroblastoma. Br J Cancer 1972;26:321–30.
58. Azuma E, Hanada M, Masuda SI, et al. Autologous tumor-specific cytotoxic T-lymphocytes in a child with neuroblastoma. Biomed Pharmacother 1990;44:487–93.
59. Macdougall IC. Could anti-inflammatory cytokine therapy improve poor treatment outcomes in dialysis patients? Nephrol Dial Transplant 2004;19(Suppl 5):V73–8.
60. Alvarez S, Blanco A, Fresno M, Muñoz-Fernández MÁ. TNF-alpha contributes to caspase-3 independent apoptosis in neuroblastoma cells: role of NFAT. PLoS One 2011;6:e16100.
61. Takei Y, Laskey R. Tumor necrosis factor alpha regulates responses to nerve growth factor, promoting neural cell survival but suppressing differentiation of neuroblastoma cells. Mol Biol Cell 2008;19:855–64.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Konno S, Yanagisawa R, Kubota N, Ogiso Y, Nishimura N, Sakashita K, et al. Investigation of patient factors associated with the number of transfusions required during chemotherapy for high-risk neuroblastoma. Vox Sang. 2021;1–9.

Received: 18 January 2021 | Revised: 22 March 2021 | Accepted: 16 April 2021

DOI: 10.1111/vox.13123

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Management of children with glucose-6-phosphate dehydrogenase deficiency presenting with acute haemolytic crisis during the SARs-COV-2 pandemic

Mohsen Elalfy[1] | Amira Adly[1] | Khadiga Eltonbary[1] | Islam Elghamry[1] | Omar Elalfy[2] | Mohamed Maebid[3] | Khaled Elsayh[4] | Heba-T-Allah N Elsayed[5] | Magdy El Ekiaby[6]

[1]Paediatrics Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[2]Complementary Medicine Department, National Research Center, Giza, Egypt

[3]Paediatrics Department, Faculty of Medicine, Beni-Suef University, Beni Suef, Egypt

[4]Paediatrics Department, Faculty of Medicine, Assuit University, Assiut, Egypt

[5]Ain Shams University Hospitals Blood Bank, Cairo, Egypt

[6]Shabrawishy Blood Bank, Giza, Egypt

Correspondence
Khadiga Yehia Elsayed Mousa Eltonbary, Ain Shams University Hospital, Cairo 11566, Egypt.
Email: khadigadoc@yahoo.com

Abstract

Background and Objectives: Shortage of blood during the severe acute respiratory syndrome-COV-2 (SARs-COV-2) pandemic impacted transfusion practice. The primary aim of the study is to assess management of acute haemolytic crisis (AHC) in glucose-6-phosphate dehydrogenase(G6PD)- deficient children during SARs-COV-2 pandemic, and then to assess blood donation situation and the role of telemedicine in management.

Methods: Assessment of G6PD-deficient children attending the Emergency Department (ER) with AHC from 1 March 2020 for 5 months in comparison to same period in the previous 2 years, in three paediatric haematology centres. AHC cases presenting with infection were tested for SARs-COV-2 using RT-PCR. Children with Hb (50–65 g/L) and who were not transfused, were followed up using telemedicine with Hb re-checked in 24 h.

Results: A 45% drop in ER visits due to G6PD deficiency-related AHC during SARs-COV-2 pandemic in comparison to the previous 2 years was observed. 10% of patients presented with fever and all tested negative for COVID-19 by RT-PCR. 33% of patients had Hb < 50 g/L and were all transfused. 50% had Hb between 50 and 65 g/L, half of them (n = 49) did not receive transfusion and only two patients (4%) required transfusion upon follow up. A restrictive transfusion strategy was adopted and one of the reasons was a 39% drop in blood donation in participating centres.

Conclusion: Fewer G6PD-deficient children with AHC visited the ER during SARs-COV-2 and most tolerated lower Hb levels. Telemedicine was an efficient tool to support their families. A restrictive transfusion strategy was clear in this study.

KEYWORDS
acute haemolytic crisis (AHC), glucose-6-phosphate dehydrogenase (G6PD), packed red blood cell transfusions (PRBCs), severe acute respiratory syndrome-COV-2 (SARs-COV-2)

INTRODUCTION

The G6PD Mediterranean mutation is the commonest mutation causing G6PD deficiency in Egypt. This variant is characterized by <10% of normal G6PD activity, making it a severe form of the disease with evident need for transfusion [1–3].

Adding G6PD deficiency to the list of screening elements in a SARs-COV-2 workup if there is a high suspicion for this genetic mutation might be recommended [4]. Triggers of red cell haemolysis are the ingestion of fava beans, infection and certain drugs as hydroxychloroquine [5, 6]. There is evidence to suggest an association between G6PD deficiency and increased susceptibility to, and severity of illness with, SARs-COV-2 infection [7, 8].

Shortage of packed red blood cell (PRBCs) transfusions in blood banks during SARs-COV-2 pandemic carries a high risk to children in need of PRBCs as emergency service [9, 10]. The threshold of red blood cell transfusions showed that there was no evidence of differences in clinical outcomes over 6 months between the critically anaemic children who received immediate transfusion and those who did not [11].

A key consideration for transfusion services is maintaining the balance between supply and demand. Donor attendance might fall, as it did by 10%–30% in the state of Washington [12] and by 30% at Canadian Blood Services (Goldman M, unpublished) [13–16].

The SARs-COV-2 crisis has made telemedicine an option that should be actively considered in most patients. Real-time telemedicine consultations reduce the time of subspecialty visits, as well as risk of infection and may thereby improve visit completion [17].

Hospitals and blood centres proactively implemented blood conservation strategies as well as efforts to maintain blood donations [18]. However, not enough studies to date have provided detailed blood usage in G6PD-deficient children during SARs-COV-2 pandemic.

In the light of such shortage, physicians were urged to reconsider the management of children with G6PD. Our national guidelines necessitates transfusion if Hb <65 to 70 g/L, but in the new approach, we have accepted lower Hb levels, guided by clinical condition and longitudinal follow-up with the use of telemedicine.

The primary objective of the study was to assess the impact of SARs-COV-2 infection on management and outcome of G6PD-deficient children presenting to ER in AHC in three haematology centres in comparison to same period in the previous 2 years.

Secondary objectives were to assess the blood donation situation and role of telemedicine in follow up of G6PD-deficient children with critical presentation during the pandemic.

METHODS

This is a prospective study to assess AHC in children with G6PD deficiency presenting to ER in three University Paediatric Haematology centres from 1 March 2020 for 5 months in comparison to same period of time in years 2019 and 2018.

Inclusion criteria

- Children with proven or highly suspected G6PD-deficiency; with or without positive family history (confirmed as G6PD-deficient 6 weeks later).
- G6PD-deficient children in AHC presented to ER in the three participating sites (exaggerated pallor and jaundice with low Hb, high both reticulocyte count and indirect serum bilirubin).
- Parental agreement to receive blood transfusion if indicated or follow up strictly/8–12 h for 48 h by video calls if not transfused.

Exclusion criteria

- AHC other than G6PD deficiency
- G6PD-deficient children not in AHC
- G6PD-deficient children whose families refused to sign the informed consent

Precipitating factors, PRBCs availability and transfusion decision were all assessed. Availability of blood and role of telemedicine in management were reported. The investigations stated in the protocol included CBCs, urine dipsticks, serum bilirubin (direct and indirect), blood grouping and cross matching. Quantitative G6PD assessment both at presentation and 4 weeks later was also included. A list of food, drugs and agents to be avoided was given to the parents. Blood bank guidelines did not approve requests for G6PD-deficient children in AHC with Hb >65 g/L if clinically stable.

Although the threshold of red blood cell transfusions for stable patients in AHC was Hb of <65 g/L, some children with Hb 50–65 g/L were not transfused either due to shortage of supply, parental worry from day of case admission during the SARs-COV-2 pandemic or following the haematologist decision of unnecessary transfusion.

In cases with Hb <50 g/L and PRBCs unavailability, the central National Blood Bank was notified and supplied such units. In case of parental fear, counselling was offered by both the paediatrician and haematologist that the benefit outweighs the minimal risk of SARs-COV-2 infection, which will be kept to a minimum after applying all necessary precautions.

Non-transfused patients were followed up, using telemedicine service (video call), every 8–12 h (for child activity, general appearance and urine colour). Providing service to re-check Hb daily for 2 days (if Hb dropped below 50 g/L or child became unstable, immediate referral for transfusion and blood was requested from the National Blood Bank). For all patients, a follow up Hb level at 24 and 48 h, G6PD assay for newly diagnosed patients and urinary dipstick every 12–24 h were done.

Educational material was given to all families of G6PD-deficient new patients. All cases presenting with evidence of infection were tested by nasal and throat swabs for SARs-COV-2 by RT-PCR [19].

Blood donation prospective data were obtained from donor attendance records, and blood inventory records between 1 March

and 31 July 2020 in comparison to retrospective data during the same period in previous 2 years. The data included the number and sources of donated blood units, and the number of released units for transfusion to AHC of G6PD-deficient children (Table 2). A recruitment text message for every potential blood donor during the SARs-COV-2 era in 2020 was sent out from May to July. Plans for blood donation campaigns to all registered providers were implemented.

Laboratory methods

- CBC was done using Coulter Counter GEM-S (Beckman Coulter Inc., Miami, Florida). Reticulocyte count was determined by Brilliant Cresyl blue stain.
- Indirect bilirubin was done on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany).
- G6PD enzyme level was assessed quantitatively using a spectrophotometer [4] at presentation to ER and 4 weeks later.
- RT-PCR using TaqPath COVID-19 CE-IVD RT-PCR Kit, 1000 reactions (Cat. No. A48067) from Thermo Fisher Scientific [20].

Telemedicine service

There has also been a significant change in the patient pathway with more being reviewed via the means of telemedicine to reduce the risk of SARs-COV-2 exposure and transmission. Laboratory test results were shared over picture archiving and communication systems to the paediatric haematologist either before or during the appointment through live video conferencing [21].

All patients' guardians of known or suspected cases of G6PD deficiency had an access to have a video call to haematologist in ER; cases with Hb <50 g/L received transfusion immediately while those with Hb 50–65 g/L were either transfused or not.

During study, telemedicine service was mainly synchronous (real-time telephone or live audio–video interaction). We ensured the availability of smartphones for all caregivers.

TABLE 1 Demographic data of G6PD-deficient children in AHC presenting to the three centres during 5 months of SARs-COV-2 pandemic in 2020 vs same period in 2018 to 2019

Variable	Year 2020 $N = 198$	Mean of year 2018 and 2019 $N = 361$	*p*-value
Age at presentation			
Months mean ± SD	(21.5 ± 8.1)	(22.3 ± 8.8)	
Range	9–48	10–50	
Male sex	178 (89.9%)	650 (mean = 325) (90%)	0.956
First episode of AHC	178 (89.9%)	635 (mean = 317) (87.95%)	0.448
Hb (g/L)			
Mean ± SD	56 ± 14	62 ± 13	<0.001
Range	2–8	2–9	
Hb <50 g/L	66 (33.3%)	90 (25%)	0.03
Hb 50–65 g/L	99 (50%)	126 (35%)	<0.001
Hb >65 g/L	33 (16.7%)	145 (40%)	<0.001
Transfused (Hb ≤50 g/L)	66/66 (100%)	90/90 (100%)	1
Non-transfused (Hb 50–65 g/L)	49/99 (49.5%)	24/126 (19%)	<0.001
PRBCs not available	19/49 (38.8%)	2/24 (8.3%)	0.007
Family decision	18/49 (36.7%)	6/24 (25%)	0.3
Haematologist decision	12/49 (24.5%)	16/24 (66.7%)	<0.001
Non-transfused Hb ≥65 g/L	33/33 (100%)	120/145 (83.0%)	0.01
Transfused	116	217	
One unit only	110/116 (94.8%)	196/217 (90.3%)	0.152
Two units	6/116 (5.1%)	21/217 (9.6%)	0.150
G6PD unit/g of Hb			
Initial			
Mean ± SD	1.6 ± 0.3	1.7 ± 0.4	0.005
Range	0.2–3.9	0.3–4.1	
6 weeks later[a]			
Mean ± SD	1.52 ± 0.32	1.61 ± 0.38	0.002

Note: $p > 0.05$: Non-significant; $p < 0.05$: Significant; $p < .01$: Highly significant.
[a]G6PD level was done in 90% initially and 100% 6 weeks later.

Contact was mainly through WhatsApp or Facebook messenger (text and video call). Asynchronous and remote patient methods were not applied.

Ethics statement

The clinical trial was conducted after approval of ethical committees of involved centres (Ain Shams, Assuit and Beni Suef Universities). Parents or guardians of all patients accepted to follow the study protocol. Parents of patients who were not transfused should be contacted regularly through video conference with the treating haematologist for fear of deterioration and were asked to respond to all queries. Parents of G6PD-deficient children with AHC accepted that strict precautions would minimize the risk of SARs-COV-2 infection.

Statistical analysis

Statistical analyses were carried out using SPSS 17.0 software package program. The comparison between two groups with qualitative data were done using Chi-square test or Fisher exact test. Independent *t*-test or Mann–Whitney test were used for two independent groups with quantitative data. Paired *t*-test or Wilcoxon Rank test were used for two paired groups with quantitative data. One way ANOVA or Kruskall-Wallis were used for more than two groups with quantitative data.

Results are considered significance if p-value < 0.05 and highly sign if <0.01.

RESULTS

Demographic data of AHC in G6PD-deficient children attending the ER in the contributing centres during the 5 months over 3 years period 2018–2020 and transfusion service provided to them are shown in Table 1.

The number of patients were stable over last 2 years with a 45% drop in ER visits due to AHC in G6PD during SARs-COV-2 pandemic. Fear of hospital visits was the primary cause of delayed presentation in more than 70%. Families did not show any concern of SAR-COV-2 being blood-transmitted, yet all were re-assured that the virus is not blood-borne.

A maximum drop of ER visits of AHC in G6PD-deficient children was observed during May as shown in Figure 1, coinciding with the peak of the pandemic in Egypt and was compensated with the maximum drop in blood donation.

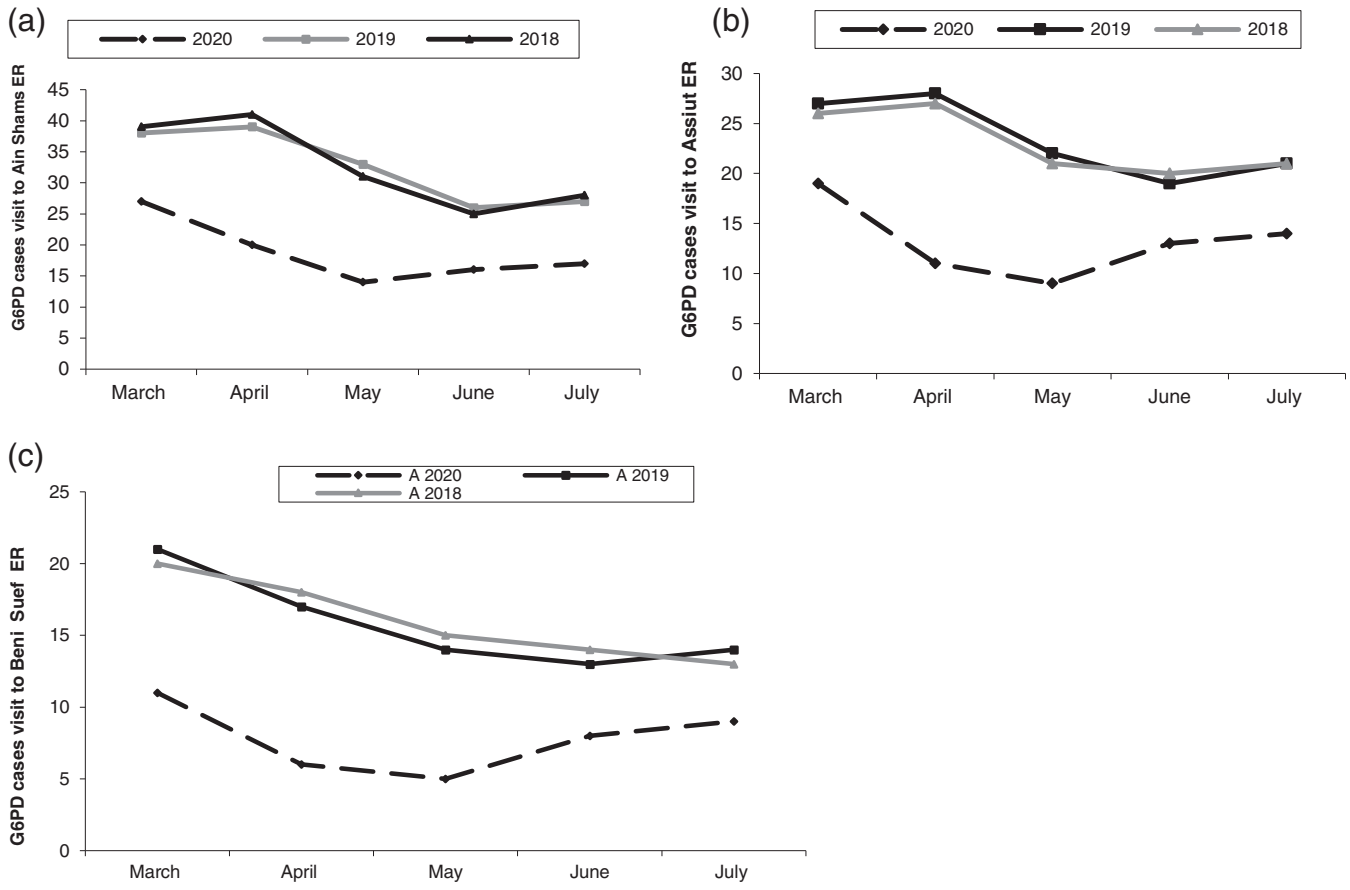


FIGURE 1 G6PD-deficient cases in AHC visits to ER in (a): Ain shams; (b) Assiut and (c) Beni Suef for 5 months period over 3 years, 2018–2020

Ingestion of fava beans and/or derivatives were the predisposing factors of haemolysis in almost 80% and offending drug intake in 10%. Only 10% of cases presented with possible infection; however none had direct contact to active SARs-COV-2 cases and all tested negative by RT-PCR swab.

The mean Hb level at presentation during the pandemic was 56 g/L, being significantly lower in comparison to previous 2 years ($p < 0.001$). Also number of patients presenting with Hb levels more than 65 g/L were significantly less than that reported over the previous 2 years. Regarding the need of transfusion, all patients presenting with Hb level less than 50 g/L were transfused as previously practiced. 100% of patients with Hb level more than 65 g/L were not transfused following a restrictive transfusion strategy during the pandemic in comparison to 83% in the previous 2 years ($p < 0.05$).

The most significant difference between COVID era and previous 2 years was in the number of non-transfused patients with Hb levels between 50 and 65 g/L. PRBC unavailability, haematologist restrictive strategy and/or parental worries were the main reasons for conservative management; however, only 4% were transfused 24 h later with the drop of Hb below 50 g/L.

Differences in transfusion rates between 2020 and previous 2 years is shown in Figure 2.

Most transfused cases received one unit but exceptionally severe cases received two units of blood in 5% during SARs-COV-2 in comparison to almost 10% in previous 2 years.

G6PD level was assessed in 90% initially and in 100% after 6 weeks from presentation or post-transfusion, a decline in level after the initial evaluation was 0%–20% (5.5%). A lower level of G6PD was detected during the pandemic.

During the outbreak of SARs-COV-2, a significant reduction in blood donations was observed in comparison to the last 2 years as shown in Table 2 and Figure 3. Females contributed less during SARs-COV-2 pandemic; however, the highly educated donors shared more. A peak reduction was noticed in May 2020 and was compensated with reduction in demand. All methods for donor recruitment were taken with a better response in late July compared to previous months coinciding with national drop of positive SARs-COV-2 cases (Figure 3). Demographic data of donors are shown in Table 2.

Regular blood donors showed 25% increase as number and double the percentage as donors compared to previous 2 years. Family replacement was less during SARs-COV-2 and even blood campaigns stopped or slowed down during April–June and re-started to peak in July (Figure 3).

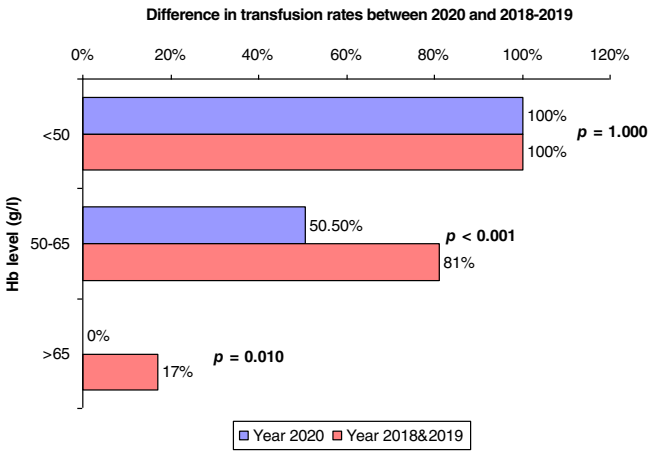


FIGURE 2 Differences in transfusion rates between 2020 and previous 2 years [Colour figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

Our results showed a marked drop in ER visits due to AHC in G6PD by 45% over 5 months during SARs-COV-2 pandemic, which could not be easily explained. The drop was observed among all severity grades of AHC; however, as expected the drop was mainly in the mild cases (75% in mild in contrast to 25% drop in severe cases) Children with mild AHC did not urge their families to visit ER.

According to consensus recommendation for red blood cell transfusion [22], the need for PRBCs does depend not only on the Hb concentration but also on the overall clinical context in addition to risks, benefits and alternatives to transfusion. The three participating centres follow a transfusion strategy of Hb level of 65 g/L or less according to the clinical condition of patient and the evidence of ongoing haemolysis.

Cases with mild AHC were all stable and were not transfused according to blood bank policy during the pandemic. Parents were assured and advised to contact the haematology team through

TABLE 2 Profile of blood donors in blood banks of the three paediatric haematology centres during March–July 2020 and 2018–2019

Blood donors	Year 2020 No = 25,989	Mean value of 2018 and 2019 No = 42,655	*p* value
Sex			
Females	2599 (10%)	8531 (20%)	<0.001
Males	23,390 (90%)	34,124 (80%)	
Age			
18–45 years	23,390 (90.0%)	38 389 (90%)	1.000
Education			
>12 years	18,192 (70%)	25,593 (60%)	<0.001
<12 years	7797 (30%)	17,062 (40%)	
Donation through			
Appointment	18,192 (70%)	12,797 (30%)	
Blood campaigns	2599 (10%)	17,062 (40%)	<0.001
Family replacement	5198 (20%)	12,796 (30%)	
Donor status			
Regular	5198 (20%)	4266 (10%)	<0.001
First timer	20,791 (80%)	38,389 (90%)	

Note: $p > 0.05$: Non-significant; $p < 0.05$: Significant; $p < 0.01$: Highly significant.

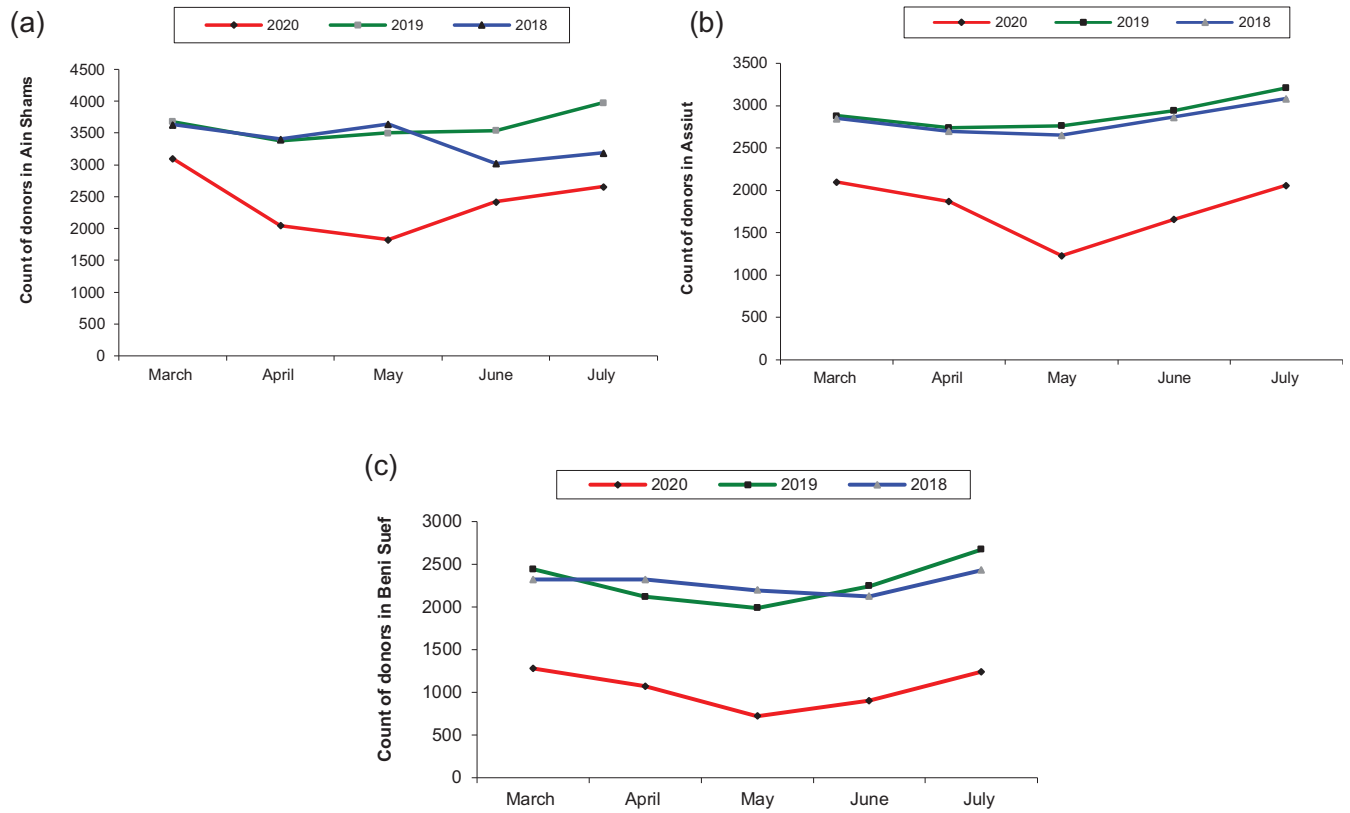


FIGURE 3 Number of donors in (a): Ain shams; (b) Assiut and (c) Beni Suef for 5 months period over 3 years, 2018–2020 [Colour figure can be viewed at wileyonlinelibrary.com]

telemedicine service if the child deteriorates. Only 10% needed to re-contact the haematologist and none were transfused during follow-up.

On the other hand, in moderately-severe cases, only 50% of them were transfused in comparison to almost 90% in the previous 2 years. All non-transfused were followed up closely. Only in 4%, Hb dropped to below 50 g/L and were transfused. This may highlight the need for more restrictive transfusion strategies in clinically stable cases as long as there is adherence to follow up.

One of the main reasons for non-transfusion was parental refusal; fear of SARs-COV-2 transmission was the main reason and not the usual fear from blood-borne infections.

Severe cases presenting to ER with Hb of 50 g/L or less were all transfused, their percentage was relatively more during the pandemic. Most of them received a single unit (95%); only 5% had received two units in contrast to almost 10% in the previous 2 years, which might be related to more restrictive transfusion policy during the SARs-COV-2 era.

Blood donation services were markedly affected by the pandemic, as reported worldwide [12], with marked reduction compared to the same period in the previous 2 years. This was secondary to the governmental restrictions issued to avoid crowding and also the fear of regular donors to visit a health care facility.

A recruitment plan started few weeks after the start of SARs-COV-2 pandemic in Egypt, mainly through social media announcements. Also text messages for regular and potential blood donors were sent out from May to July, with a significant rise in July coinciding with significant drop in reported SARs-COV-2 cases.

Regular blood donors played a great role during SARs-COV-2 time, not only they showed 25% increment in number in comparison to previous 2 years but also had higher Hb levels (data were not shown) which was mostly due to repeated advice to consume more haeme iron [23]. Surprisingly, family replacement was less during SARs-COV-2 and even blood campaigns either stopped or was less frequent through April–June and re-started to peak in July. Females contributed less during SARs-COV-2 pandemic; however, the well educated donors shared more.

Balance between shortage of PRBCs and unnecessary transfusion should be maintained well. Restrictive transfusion for stable moderate AHC cases due to G6PD deficiency should be studied more in a prospective study in unprivileged countries with shortage of blood donation. The possibility of using the same model after control of SARs-COV-2 pandemic should be well assessed.

The fate of missed cases of G6PD-deficient children with AHC who did not attend to ER to seek medical advice was unknown. We did our best to clear our retrospective data to be suitable for comparison with the prospective data. SARs-COV-2 RT-PCR screening was done only in 10% of cases who presented with evidence of infection; all were negative and chest CT was not done; however, asymptomatic

cases might be possibly missed. Telemedicine was done only for non-transfused critical cases as well as any one requesting the service, meanwhile a critical evaluation should be done in case of refusal of ER visit for border-line cases.

Telemedicine channels as Whats app and Facebook do not protect patient's privacy and personal data protection.

In conclusion, during the COVID-19 pandemic and with shortage of blood supply, a more restrictive transfusion practice for AHC in G6PD-deficient children was implemented compared to the previous 2 years. A delay in presentation to ER was noticed. It was clear that home supervision through telemedicine was a good option. Maximizing the role of regular blood donors is mandatory in Egypt and restrictive transfusion strategy should be assessed better in the future.

ACKNOWLEDGEMENTS

No funding was secured for this study.

M.E. is responsible for the design of the work and contributed along with all the remaining authors to:

1. The acquisition, analysis or interpretation of data for the work;
2. Drafting the work and revising it critically for important intellectual content;
3. Final approval of the version to be published;
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Khadiga Eltonbary https://orcid.org/0000-0002-6519-0840
Heba-T-Allah N Elsayed https://orcid.org/0000-0002-9463-0219
Magdy El Ekiaby https://orcid.org/0000-0002-3881-1605

REFERENCES

1. Arnaout HH, El-Gharbawy NM, Shaheen IA, Afifi RA, EL-Dayem OYA. Incidence and association of 563 C/T mediterranean and the silent 1311C/T G6PD mutations in G6PD-deficient Egyptian children. Lab Med. 2011;42:355–60.
2. Settin A, Al-Haggar M, Al-Baz R, Yousof H, Osman N. Screening for G6PD Mediterranean mutation among Egyptian neonates with high or prolonged jaundice. HAEMA. 2006;9:83–90.
3. Osman HG, Zahran FM, El-Sokkary AMA, El-Said A, Sabry AM. Identification of Mediterranean mutation in Egyptian favism patients. Eur Rev Med Pharmacol Sci. 2014;18:2821–7.
4. Jamerson BD, Haryadi TH, Bohannon A. Glucose-6-phosphate dehydrogenase deficiency: an actionable risk factor for patients with COVID-19? Arch Med Res. 2020;51:743–4.
5. Luzzatto L, Arese P. Favism and glucose-6-phosphate dehydrogenase deficiency. N Engl J Med. 2018;378:60–71.
6. Beauverd Y, Adam Y, Assouline B, Samii K. COVID-19 infection and treatment with hydroxychloroquine cause severe haemolysis crisis in a patient with glucose-6-phosphate dehydrogenase deficiency. Eur J Haematol. 2020;105:357–9.
7. Vick DJ. Glucose-6-phosphate dehydrogenase deficiency and COVID-19 infection. Mayo Clin Proc. 2020;95:1803–4.
8. Wu YH, Tseng CP, Cheng ML, Ho HY, Shih SR, Chiu DTY. Glucose-6-phosphate dehydrogenase deficiency enhances human coronavirus 229E infection. J Infect Dis. 2008;197:812–6.
9. Stanworth SJ, New HV, Apelseth TON, Brunskill S, Cardigan R, Doree C, et al. Effects of the COVID-19 pandemic on supply and use of blood for transfusion. Lancet Haematol. 2020;7:e756–64.
10. Arcot PJ, Kumar K, Mukhopadhyay T, Subramanian A. Potential challenges faced by blood bank services during COVID-19 pandemic and their mitigative measures: the Indian scenario. Transfus Apher Sci. 2020;59:102877.
11. Maitland K, Kiguli S, Olupot-Olupot P, Engoru C, Mallewa M, Saramago Goncalves P, et al. Immediate transfusion in African children with uncomplicated severe anemia. N Engl J Med. 2019;381:407–19.
12. Pagano MB, Hess JR, Tsang HC, Staley E, Gernsheimer T, Sen N, et al. Prepare to adapt: blood supply and transfusion support during the first 2 weeks of the 2019 novel coronavirus (COVID-19) pandemic affecting Washington State. Transfusion. 2020;60:908–11.
13. Franchini M, Farrugia A, Velati C, Zanetti A, Romanò L, Grazzini G, et al. The impact of the SARS-CoV-2 outbreak on the safety and availability of blood transfusions in Italy. Vox Sang. 2020;115:603–5.
14. Mohammadi S, Tabatabaei Yazdi SM, Eshghi P, Norooznezhad AH. Coronavirus disease 2019 (COVID-19) and decrease in blood donation: experience of Iranian Blood Transfusion Organization (IBTO). Vox Sang. 2020;115:595–6.
15. Wang Y, Han W, Pan L, Wang C, Liu Y, Hu W, et al. Impact of COVID-19 on blood centres in Zhejiang province China. Vox Sang. 2020;115:502–6.
16. Chang L, Yan Y, Wang L. Coronavirus disease 2019: coronaviruses and blood safety. Transfus Med Rev. 2020;34:75–80.
17. Bashiri M, Greenfield LJ, Oliveto A. Telemedicine interest for routine follow-up care among neurology patients in Arkansas. Telemed e-Health. 2016;22:514–8.
18. Shander A, Goobie SM, Warner MA, Aapro M, Bisbe E, Perez-Calatayud AA, et al. Essential role of patient blood management in a pandemic: a call for action. Anesth Analg. 2020;131:74–85.
19. Wu J, Liu J, Li S, Peng Z, Xiao Z, Wang X, et al. Detection and analysis of nucleic acid in various biological samples of COVID-19 patients. Travel Med Infect Dis. 2020;37:101673.
20. Kassem AM, Talaat H, Shawky S, Fouad R, Amer K, Elnagdy T, et al. SARS-CoV-2 infection among healthcare workers of a gastroenterological service in a tertiary care facility. Arab J Gastroenterol. 2020;21:151–5.
21. Putora PM, Baudis M, Beadle BM, El Naqa I, Giordano FA, Nicolay NH. Oncology informatics: status quo and outlook. Oncology. 2020;98:329–31.
22. Valentine SL, Bembea MM, Muszynski JA, Cholette JM, Doctor A, Spinella PC, et al. Consensus recommendations for rbc transfusion practice in critically ill children from the pediatric critical care transfusion and anemia expertise initiative. Pediatr Crit Care Med. 2018;19:884–98.
23. Timmer TC, de Groot R, Rijnhart JJM, Lakerveld J, Brug J, CWM P, et al. Dietary intake of heme iron is associated with ferritin and hemoglobin levels in Dutch blood donors: results from donor InSight. Haematologica. 2019;105:2400–6.

How to cite this article: Elalfy M, Adly A, Eltonbary K, et al. Management of children with glucose-6-phosphate dehydrogenase deficiency presenting with acute haemolytic crisis during the SARs-COV-2 pandemic. Vox Sang. 2021;1–7.

Received: 27 March 2021 | Revised: 20 April 2021 | Accepted: 1 May 2021

DOI: 10.1111/vox.13132

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

A randomized study of a best practice alert for platelet transfusions

Colin Murphy[1] | Eric Mou[2] | Emily Pang[3] | Lisa Shieh[4] | Jason Hom[4] | Neil Shah[1]

[1]Division of Transfusion Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, California, USA

[2]Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA

[3]Stanford University School of Medicine, Stanford, California, USA

[4]Division of Hospital Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA

Correspondence
Colin Murphy, Division of Transfusion Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA.
Email: colin.murphy@som.umaryland.edu

Abstract

Background and Objectives: Inappropriate platelet transfusions represent an opportunity for improvements in patient care. Use of a best practice alert (BPA) as clinical decision support (CDS) for red cell transfusions has successfully reduced unnecessary red blood cell (RBC) transfusions in prior studies. We studied the impact of a platelet transfusion BPA with visibility randomized by patient chart.

Materials and Methods: A BPA was built to introduce CDS at the time of platelet ordering in the electronic health record. Alert visibility was randomized at the patient encounter level. BPA eligible platelet transfusions for patients with both visible and non-visible alerts were recorded along with reasons given for override of the BPA. Focused interviews were performed with providers who interacted with the BPA to assess its impact on their decision making.

Results: Over a 9-month study period, 446 patient charts were randomized. The visible alert group used 25.3% fewer BPA eligible platelets. Mean monthly usage of platelets eligible for BPA display was 65.7 for the control group and 49.1 for the visible alert group ($p = 0.07$). BPA-eligible platelets used per inpatient day at risk per month were not significantly different between groups (2.4 vs. 2.1, $p = 0.53$).

Conclusion: It is feasible to study CDS via chart-based randomization. A platelet BPA reduced total platelets used over the study period and may have resulted in $151,069 in yearly savings, although there were no differences when adjusted for inpatient days at risk. During interviews, providers offered additional workflow insights allowing further improvement of CDS for platelet transfusions.

KEYWORDS
clinical trial, patient blood management, platelet components, platelet transfusion, transfusion therapy

INTRODUCTION

Platelet transfusions are an effective haemostatic therapy for bleeding thrombocytopenic patients [1, 2]. Multiple trials have examined appropriate thresholds for prophylactic use of platelets, predominantly in stable cancer patients [3–6]. Guidelines for platelet thresholds in other clinical situations, including invasive procedures and WHO grade 2 or worse bleeding, have been established by several professional organizations and national institutes [7–9].

Despite publication and promotion of these guidelines, clinical practices are not always consistent with the accumulated evidence [10, 11]. One intervention to increase compliance with guidelines is

clinical decision support (CDS) using a best practice alert (BPA) that requires provider interaction during orders placed in the electronic health record (EHR). As part of a comprehensive patient blood management (PBM) programme, implementation of BPAs for red blood cell (RBC) transfusion utilizing evidence-based guidelines [12] has been associated with reduced red cell usage and better patient outcomes [13, 14]. A similar effort targeting plasma ordering based on International Standardized Ratio (INR) also showed efficacy in reducing potentially inappropriate usage [15].

Strategies aimed at targeting inappropriate platelet transfusion are more challenging relative to RBCs or plasma as the transfusion threshold is variable for different patient conditions. One previous effort to decrease platelet utilization employed targeted staffing interventions [16]. CDS for platelet utilization has a mixed record [17], with multiple studies showing no difference in platelet utilization or rate of BPA triggering after implementation of a BPA for platelet transfusion [18–20]. At our institution, we sought to decrease overall platelet utilization via a targeted BPA advising providers at the time of order placement to remove potentially inappropriate orders. Importantly, we built our intervention to be tested in a randomized fashion in contrast to previous studies of CDS in BM.

MATERIALS AND METHODS

A targeted BPA was built to introduce CDS at the time of platelet ordering within our EHR (Epic Systems Corp. Verona, W.I.). The BPA triggered on a platelet order whenever the patient's most recent platelet count was greater than 50 k/μl with a maximum lookback time of 48 h for the count value. There were exclusion criteria built to avoid targeting the wrong patients or providers. The BPA was excluded from all procedural areas (including the emergency department, operating rooms, interventional radiology, and interventional cardiology), as part of emergency or massive transfusion, and for all neurosurgery providers.

The BPA was activated in the adult inpatient hospital setting of a large, tertiary care academic medical centre on May 9th, 2019 and run through February 4th, 2020. Alerts were randomized at the patient encounter level to trigger either in standard or background (control) fashion. For silent alerts, no BPA was shown on-screen, but the occurrence was recorded. For standard alerts, the BPA appeared on-screen, informing the provider that their platelet transfusion order was potentially inappropriate and citing supportive evidence (Figure 1). Providers had the option of following or overriding the alert, but for the latter a pre-specified list of clinical justifications or 'Other' needed to be selected. Providers who chose 'Other' could enter a free text justification but this was not required. Pre-specified options included upcoming neurosurgery, intracranial haemorrhage cardiac bypass surgery, known qualitative platelet defects, or patients taking antiplatelet drugs. If a user selected any override reason besides 'Other', such as neurosurgery, the alert was subsequently not triggered for ANY user for that patient encounter for a 36-h duration. If platelet defect was selected, a longer lockout period of 72 h was enabled. This type of lockout was designed to prevent alert fatigue such that if there was a clinical justification for transfusion, the same or subsequent user would not continue to see the alert during a likely affected time period.

For patients eligible for the BPA, total platelet orders and those placed for patients with platelet counts over 50 k/μl were evaluated independently of the BPA using silent alerts that triggered with every completed platelet order, recording the date and number of platelets ordered. Analyses were limited to regular platelet orders and excluded platelet orders as part of emergency or massive transfusion. BPA-eligible platelets were defined as platelets issued within 24 h of a visible or control (silent) alert.

Estimated per unit costs were based on charges for platelet transfusions as listed in the hospital charge master. Data cleaning, combination, and analysis were performed in the R statistical computing language version 4.0.2 ('Taking Off Again') utilizing the tidyr and ggplot2 packages [21–23]. In univariate comparisons, *p* values are reported for continuous variables as the result of

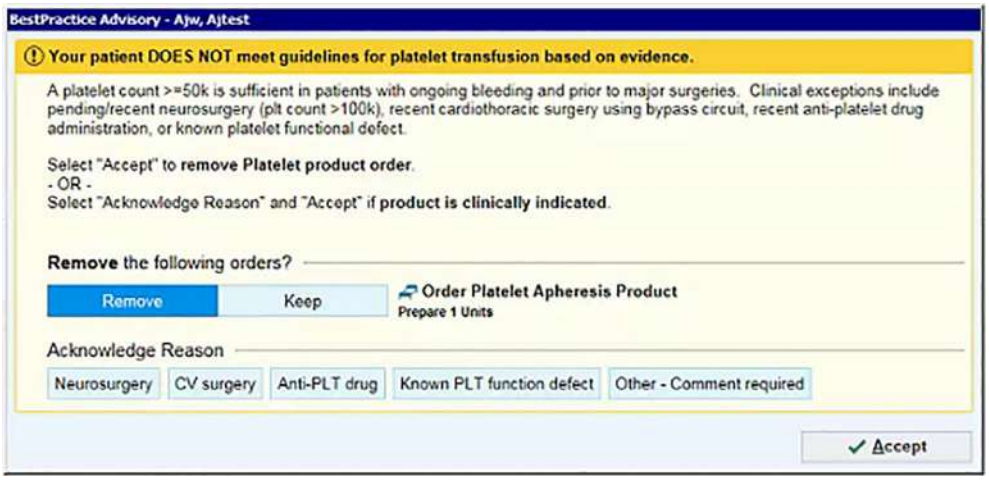


FIGURE 1 Best practice alert (BPA) appearance in the electronic health record [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary data for all hospitalized patients receiving at least one platelet during the study period, 5/10/19–2/4/20

Category	Total patients	Platelets issued
All transfused patients	1331	5336
Study patients	446	1033
Control alert	218	591
Visible alert	228	442

Note: All transfused patients category includes patients not meeting best practice alert (BPA) criteria for enrolment.

TABLE 2 Demographic characteristics for transfused patients

	Control alert	Visible alert
Transfused patients	204	201
Sex, male	123 (60%)	130 (65%)
Age	62 (49.75–72)	60 (51–70)
Primary service		
Critical care/Anaesthesia	36%	30%
Internal medicine	24%	31%
Cardiothoracic surgery	15%	16%
General surgery	11%	10%
Emergency medicine	8%	7%
Other	6%	7%

Note: Age is presented as median and interquartile range. Primary team denoted is based on clinical association of provider ordering platelets. Other category includes neurology, neurosurgery, orthopaedic surgery and obstetrics.

two-sided Student's t tests with a significance threshold of 0.05, unless specified otherwise.

The free-text override comments were independently reviewed by two study authors (E.M. and E.P.) to assess provider sentiment to alert guidance. Comments were tagged into a set of general categories by iterative inductive analysis of the comment content, and each comment could be assigned multiple tags. Inter-rater variability was assessed by calculating a Cohen's kappa coefficient. These tagged comment categories were then summed and plotted as percentage of all free-text override reasons to assess common reasons for bypass not included in pre-selected options.

In addition, focused interviews were conducted in a subset of providers who interacted with the visible alert in order to gain insight into their approach to ordering platelets and experiences with interacting with the BPA. Iterative inductive analysis was performed to identify recurring themes across participants. A $10 (USD) gift card was offered as an incentive for provider participation. This study was approved by the Stanford Institutional Review Board.

RESULTS

Over the 9-month study period, 5336 platelets were issued to 1331 patients in orders of two or less platelets. A total of 218 patient charts were randomized to the control alert, and 228 were randomized to the standard visible alert. Over the study period, the control group used 591 BPA-eligible platelets and the visible alert group used 442 BPA-eligible platelets, a 25.3% reduction (Table 1). In total, 204 patients in the control arm and 201 patients in the visible alert arm were transfused (Table 2). Mean monthly usage of platelets eligible for BPA display was 65.7 for the control group and 49.1 for the visible alert group ($p = 0.07$). BPA-eligible platelets used per inpatient day at risk per month were not significantly different between groups (2.4 vs. 2.1, $p = 0.53$; Table 3; Figure 2).

TABLE 3 Monthly best practice alert (BPA) eligible platelet usage and platelets per inpatient day (PPID) at risk by alert visibility for study patients

	Platelets	PPID
Control alert	65.7 (20.8)	2.3 (0.8)
Visible alert	49.1 (13.5)	2.1 (0.8)
p Value	0.07	0.53

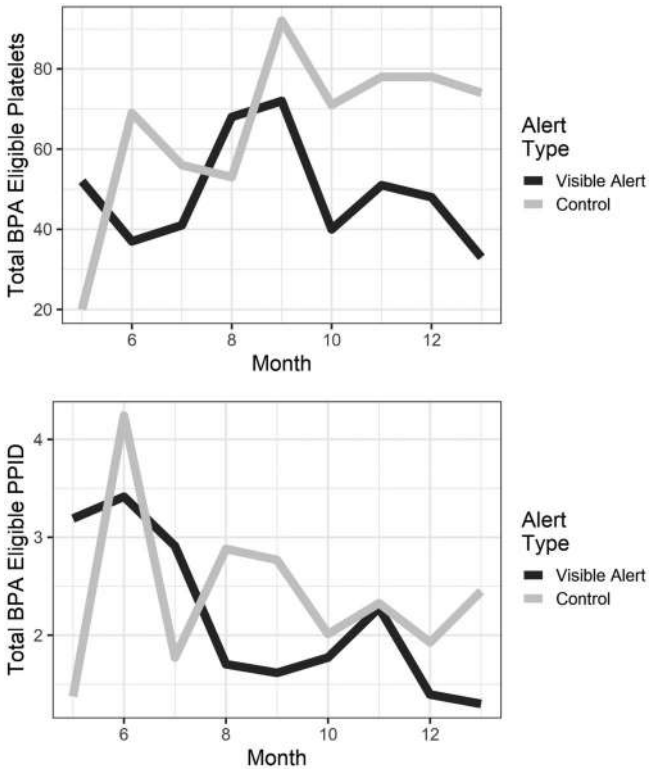


FIGURE 2 Monthly usage for best practice alert (BPA) eligible platelets by alert visibility for (A) total platelets and (B) platelets per inpatient day at risk (PPID) during the study period of 5/9/19 and 2/4/20

In the visible alert arm, 353 alerts were generated, of which 327 were overridden. Cardiovascular bypass and platelet function defects were the most common pre-selected descriptive reasons given for alert bypass, followed by neurosurgical procedures and active CNS bleeding (Figure 3). The total of 149 orders were

overridden for 'Other' reasons, with resultant free-text justification provided in 133 cases (89% user feedback rate). These comments were subsequently tagged into distinct categories with a Cohen's kappa coefficient of 0.911 ($p < 0.001$) between raters. Figure 4 displays the different categories into which override comments were classified. The most commonly provided free-text override comments included platelets ordered in the pre-operative/pre-procedural setting, patients with active bleeding (non-CNS and CNS), and those harbouring qualitative platelet defects.

Twenty-four providers participated in interviews, which focused on each individual's approach to platelet transfusions and feedback on their interaction with the BPA. Major themes and representative excerpts are shown in Table 4. Iterative inductive analysis of interview transcripts revealed two major themes: reasons why providers ordered platelet transfusions despite the appearance of the BPA advising against it and feedback on how to improve the compliance with and education derived from these alerts.

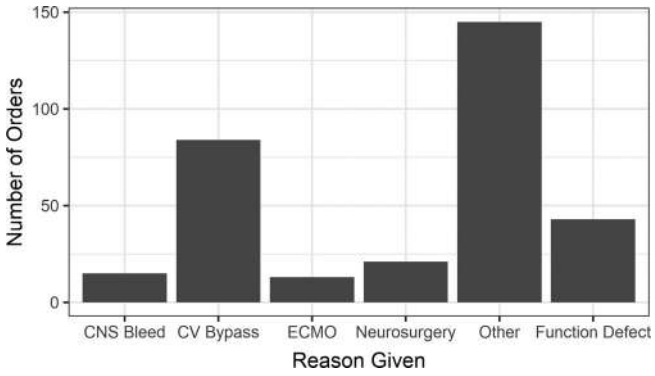


FIGURE 3 Totals of selected reasons given for bypass of the best practice alert (BPA) at time of order placement. CNS, central nervous system; CV, cardiovascular; ECMO, extracorporeal membrane oxygenation

DISCUSSION

Over 9 months, patients with a visible BPA in their chart used fewer total platelets, although the monthly difference was not significant. Additionally qualifying this finding, there was no difference in usage when adjusted for total inpatient days at risk in each arm. Total platelet usage in the alert group was decreased 25.3% as compared to the control group. This represents $111,750 cost difference potentially attributable to the intervention with an average product purchase price/supply price of ~$750. Annualized, this would be $151,069 in potential yearly savings. In addition, the total costs of transfusion can be up to 3–5 times purchase or supply costs, suggesting greater unmeasured impacts on cost savings [24].

Monthly platelet usage did not significantly vary between randomized groups in any of the categories evaluated. Lower frequency and increased variability of platelet usage by month as compared to red cell usage makes establishing statistical significance for changes in platelet transfusion practice more challenging. Future studies of platelet transfusion practice modifications could include additional indices of patient volume and severity to provide better adjusted statistical comparisons between trial arms. Evaluating other aspects of blood bank operations for impact of the BPA beyond total blood product usage could also be helpful. For red cells, a study evaluating CDS found no significant effect on compliance with CDS alone but did find a sharp reduction in products dispensed and returned with a combination of CDS and remote electronic issuance [25]. Evaluating the impact of a platelet transfusion CDS could include assessing whether decreased platelet transfusions resulted in less need to maintain a large platelet inventory, potentially leading to adjusted par levels and reducing platelet waste. Other potential targets for evaluation include patient centred outcomes such as in-hospital mortality, length of stay, and number of transfusion reactions [17].

The vast majority of BPA alerts were overridden, similar to other studies of transfusion CDS [26]. However, while the BPA may not have been heeded or applicable in individual instances, its presence may have a more general effect of modifying subsequent provider behaviours regarding platelet usage and medically addressing

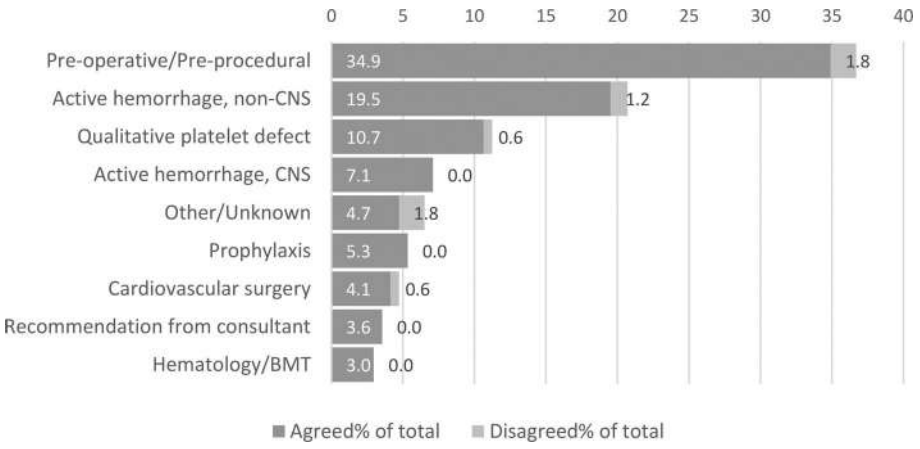


FIGURE 4 Categorization of free-text override comments. Override comments were reviewed by two reviewers who indicated agreement or disagreement with the selected reason. CNS, central nervous system; BMT, bone marrow transplant

TABLE 4 Themes emerging from providers' focused interviews

Why do providers transfuse despite BPA or guideline recommendations?	How can we improve the implementation of this BPA?
Theme 1: Additional instructions on platelet ordering from others	*Theme 1: Need for context-specific implementation*
'Decisions are rarely unilateral in these situations and it can be hard to follow the BPA if it requires opposing the recommendation from someone higher up in the medical hierarchy'.	'If the patient is stable, everyone is comfortable with an evidence-based best practice. But it is different when the patient is dynamic and you need to think preemptively'.
'It can be tough to go along with the recommendation if the service wants something different'.	'In emergency situations, the person placing the order is rushing and does not have the time to look at the BPA carefully'.
'If we are deviating from known cutoffs, it is usually because someone has told us to'.	'The setting matters. It is not appropriate to have this BPA fire for every single service since thresholds vary'.
'On night coverage, we are unlikely to overturn a transfusion recommendation suggested by the day team'.	
Theme 2: Acute or emergent patient situations	*Theme 2: Minimize alert fatigue*
'In an emergent bleeding situation, aggressively and quickly stabilizing the patient takes precedence over all else'.	'For services where there is great familiarity with the clinical context and the ordering platelets, this alert can be very irritating.'
	'It would be frustrating if the alert fires multiple times on the same patient who is actively bleeding'.
'When the patient dynamics change, we begin to follow our intuitive experience. It's a lot harder then to follow evidence-based best practice that is likely based on a static patient model'.	'Brevity is important. Too many required clicks lead to user frustration'.
Theme 3: Lack of Awareness of or Non-adherence to Existing Guidelines	*Theme 3: Need to Reach All Members of Healthcare Team and Influence Those with Ordering Power*
'I work in the hematology department, and I wasn't aware of any guidelines until now'.	'It's important to identify and educate whoever recommended the procedure, as these 'outliers' are likely responsible for the greatest burden of inappropriate platelet transfusions'.
'We need to target he intervention at services that are less familiar with platelet transfusions and frequently violating the guidelines'.	'It's important to have attendings on board with following these guidelines'.
'I think most conflict comes from services that set differing goals for themselves'.	
Theme 4: Preventative nature of blood transfusions	
'I see most violations in situations where we are trying to do anything to support the patient'.	
'Ordering platelets can come from a place of fear, where we place the order even if there is an alert because there is a chance the patient may benefit'.	
'When a patient destabilizes, you preemptively think about what could be the worst-case outcome for the patient'.	

thrombocytopenia. Previous studies of BPAs targeting one blood product have found trends towards decreases in others, suggesting that providers trained to think about blood transfusion in one context will apply that training in others [13].

Even though the alert was constructed in such a manner as to avoid triggering in patients undergoing selected indications like cardiovascular bypass, extracorporeal membrane oxygenation (ECMO) or neurosurgery, the most common bypass reasons included these categories. This illustrates the importance of building frequently encountered scenarios into a BPA, as well as evaluating the efficacy of location specific BPA targeting. Additionally, analyses of the free-text justifications for alert override demonstrate that there exists significant nuance in certain patient scenarios that may warrant transfusion despite a numerical platelet count that suggests otherwise.

A frequently cited reason for override in our analysis included patients in the pre-operative/pre-procedural setting. Though we were unable to discern the specific operation that was anticipated, it is understandable that depending on the severity of illness for which the patient was admitted, combined with a potentially major upcoming surgery, the ordering provider felt the use of prophylactic platelet transfusion was justified regardless of platelet count. Similarly, patients experiencing active haemorrhage or qualitative platelet defects may have prompted transfusions in an effort to stem this bleeding, depending on their accompanying comorbidities and clinical trajectory at the time. An additional consideration is that providers may choose clinical override justifications that are not appropriate for the patient, as there is no continuous process to verify the accuracy of the override reason. Taken together, future interventions seeking a more tailored approach should attempt to account for the complexity of certain admission diagnoses or intended diagnostic/therapeutic interventions.

Focused interviews revealed several interesting themes that may further explain reasons for transfusion despite the recommendation

of our BPA. Suboptimal utilization of evidence-based guidelines, due to both providers' lack of awareness and preference for department-based practice patterns, was cited as driving transfusion orders despite an opposing BPA recommendation. Uncertain patient situations, especially in those with hemodynamic instability and suspected bleeding, also prompted providers to transfuse platelets at a lower threshold. In addition, the hierarchical and multi-disciplinary nature of patient care appeared to influence some providers' practices, with some citing attending physicians or other consultants as the reason for placing these orders. Overall, these findings not only highlight the importance of ongoing educational efforts to improve awareness of best practices, but also the challenges posed by patient acuity and interpersonal dynamics in adopting these behaviours. Prior studies have similarly identified these themes as reasons why BPAs are not consistently followed [26].

Recognizing these challenges, providers offered suggestions for improving the effectiveness of the BPA alert that centred on three major themes. First, context matters. Though the educational intent of the intervention was generally appreciated, many felt that the value diminished in the setting of a decompensating patient, where emergent aspects of the situation at-hand often prompted transfusions regardless of guideline recommendations. A BPA deployed in these settings created the opposite effect by breeding frustration and dissatisfaction with the extra 'clicks' required to complete the transfusion order. Additionally, providers cited the unequal influence of certain care team members in determining ordering practices, including individuals with entrenched ordering patterns, or attending physicians who wielded the ultimate deciding power to transfuse or not.

Taken together, these insights suggest that future BPA efforts could be improved if they are context-specific, dynamically responsive to the practice patterns of the ordering provider, and visible to all ranks and levels of the healthcare team. Although we designed our CDS to not trigger in procedural settings, additional sophistication could avoid CDS triggering for patients who recently had cardiopulmonary bypass, a commonly selected bypass reason (Figure 3). Addressing the interview theme that ordering providers were not always those with the most authority to make decisions about the appropriateness of platelet therapy, displaying the CDS to more members of the care team via pathways other than the EHR could potentially be impactful. This would also satisfy more of the Agency for Healthcare Research and Quality's CDS Five Rights, providing the right information to the right person via the right channel [27].

We believe the most significant part of this effort is to show that technological tools for PBM such as alerts can be studied in a systematic and scientifically sound fashion through randomization to prove causation as opposed to relying on historical controls or cluster randomization by location or provider. Routine randomization of CDS can help organizations evaluate which interventions are effective in real-world practice [28]. Studying provider responses can assist in refining CDS in a real-time and interactive fashion.

However, technological tools such as alerts and other decision supports are not a panacea in aligning clinical practice to scientific evidence. In the modern medical centre, they must be built with an appreciation for complex decision making in a multi-role specialty health system, with mechanisms to incorporate provider engagement and feedback. Done correctly, technological tools can help change provider behaviour regarding transfusions and with a much lower input of resources than traditional interventions.

ACKNOWLEDGEMENTS

CM and NS conceived of and developed the research idea. NS built the best practice alert, validated the randomization and revised the manuscript. CM performed the total platelet usage data analysis, wrote and revised the manuscript. JH and LS assisted in implementation of the alert and revised the manuscript with focus on best practice alerts. EM and EP performed the physician surveys, analysed the results and wrote the discussion surrounding provider feelings on the best practice alert. All authors revised and reviewed the final manuscript.

DATA AVAILABILITY STATEMENT

Data cannot be shared for this manuscript, which involves patient specific data, due to the specific requirements of the IRB approval and HIPAA privacy rules for patient identifying information.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

FUNDING INFORMATION

None.

ORCID

Colin Murphy https://orcid.org/0000-0002-1887-2646

REFERENCES

1. Duke WW. The relation of blood platelets to hemorrhagic disease. JAMA. 1983;250:1201–9.
2. Freireich EJ. Origins of platelet transfusion therapy. Transfus Med Rev. 2011;25:252–6.
3. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. N Engl J Med. 1962;266:905–9.
4. Slichter SJ, Harker LA. Thrombocytopenia: Mechanisms and management of defects in platelet production. Clin Haematol. 1978;7:523–39.
5. Rebulla P, Finazzi G, Marangoni F, et al. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukemia. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. N Engl J Med. 1997;337:1870–5.
6. Slichter SJ, Kaufman RM, Assmann SF, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med. 2010;362:600–13.
7. National Clinical Guideline Centre (UK). Blood transfusion. National Institute for Health and Care Excellence (UK); 2015 https://www.ncbi.nlm.nih.gov/books/NBK338783/, Accessed March 27, 2021.
8. Schiffer CA, Bohlke K, Delaney M, et al. Platelet transfusion for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2018;36:283–99.

9. Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: A clinical practice guideline from the AABB. Ann Intern Med. 2015;162:205–13.
10. Greeno E, Mccullough J, Weisdorf D. Platelet utilization and the transfusion trigger: A prospective analysis. Transfusion. 2007;47:201–5.
11. Shander A, Fink A, Javidroozi M, et al. Appropriateness of allogeneic red blood cell transfusion: The international consensus conference on transfusion outcomes. Transfus Med Rev. 2011;25:232–246.e53.
12. Carson JL, Guyatt G, Heddle NM, et al. Clinical practice guidelines from the AABB: Red blood cell transfusion thresholds and storage. JAMA. 2016;316:2025–35. https://doi.org/10.1001/jama.2016.9185.
13. Goodnough LT, Shieh L, Hadhazy E, Cheng N, Khari P, Maggio P. Improved blood utilization using real-time clinical decision support. Transfusion. 2014;54:1358–65.
14. Goodnough LT, Maggio P, Hadhazy E, et al. Restrictive blood transfusion practices are associated with improved patient outcomes. Transfusion. 2014;54:2753–9.
15. Shah N, Baker SA, Spain D, et al. Real-time clinical decision support decreases inappropriate plasma transfusion. Am J Clin Pathol. 2017; 148:154–60.
16. Sekhar M, Clark S, Atugonza R, Li A, Chaudhry Z. Effective implementation of a patient blood management programme for platelets. Transfus Med. 2016;26:422–31.
17. Hibbs SP, Nielsen ND, Brunskill S, et al. The impact of electronic decision support on transfusion practice: A systematic review. Transfus Med Rev. 2015;29:14–23.
18. Butler CE, Noel S, Hibbs SP, et al. Implementation of a clinical decision support system improves compliance with restrictive transfusion policies in hematology patients. Transfusion. 2015;55:1964–71.
19. Pentti J, Syrjälä M, Pettilä V. Computerized quality assurance of decisions to transfuse blood components to critically ill patients. Acta Anaesthesiol Scand. 2003;47:973–8.
20. Collins RA, Triulzi DJ, Waters JH, Reddy V, Yazer MH. Evaluation of real-time clinical decision support systems for platelet and cryoprecipitate orders. Am J Clin Pathol. 2014;141:78–84.
21. R Core Team. R: A language and environment for statistical computing. R Foundation; 2019. https://www.R-project.org/.
22. Wickham H, Henry L. tidyr: Easily Tidy Data with 'spread' and 'gather' Functions. R package version 0.8.3; 2019. https://CRAN.R-project.org/package=tidyr
23. Wickham H. ggplot2: Elegant graphics for data analysis. Springer-Verlag; 2016.
24. Shander A, Hofmann A, Ozawa S, Theusinger OM, Gombotz H, Spahn DR. Activity-based costs of blood transfusions in surgical patients at four hospitals. Transfusion. 2010;50:753–65.
25. Hibbs SP, Noel S, Miles D, Staves J, Murphy MF. The impact of electronic decision support and electronic remote blood issue on transfusion practice. Transfus Med. 2014;24:274–9.
26. Chen JH, Fang DZ, Tim Goodnough L, Evans KH, Lee Porter M, Shieh L. Why providers transfuse blood products outside recommended guidelines in spite of integrated electronic best practice alerts. J Hosp Med. 2015;10:1–7.
27. Clinical Decision Support (CDS). Agency for Healthcare Research and Quality website. https://digital.ahrq.gov/ahrq-funded-projects/current-health-it-priorities/clinical-decision-support-cds/chapter-1-approaching-clinical-decision/section-2-overview-cds-five-rights, Accessed April 20, 2021
28. Horwitz LI, Kuznetsova M, Jones SA. Creating a learning health system through rapid-cycle, randomized testing. N Engl J Med. 2019; 381:1175–9.

How to cite this article: Murphy C, Mou E, Pang E, Shieh L, Hom J, Shah N. A randomized study of a best practice alert for platelet transfusions. Vox Sang. 2021;1–7.

Received: 1 March 2021 | Revised: 8 April 2021 | Accepted: 11 April 2021

DOI: 10.1111/vox.13121

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Frequencies of glycophorin variants and alloantibodies against Hil and MINY antigens in Japanese

Sayaka Kaito[1] | Yumi Suzuki[1] | Atsuko Masuno[1] | Kazumi Isa[2] | Chizu Toyoda[1] | Takayuki Onodera[1] | Kenichi Ogasawara[2] | Makoto Uchikawa[1] | Nelson-Hirokazu Tsuno[1] | Masahiro Satake[2]

[1]Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Tokyo, Japan

[2]Japanese Red Cross Central Blood Institute, Tokyo, Japan

Correspondence
Kenichi Ogasawara, Japanese Red Cross Central Blood Institute, 2-1-67 Tatsumi, Koto-ku, Tokyo 135-8521, Japan
Email: k-ogasawara@jrc.or.jp

Abstract

Background and Objectives: Antigens of the MNS blood group system are expressed on the red blood cell (RBC) membrane on glycophorin A (GPA) and glycophorin B (GPB) or on hybrid molecules of GPA and GPB. This study investigated the distribution of glycophorin variants and alloantibodies against Hil and MINY among Japanese individuals.

Methods: Mi(a+) or Hil+ RBCs were screened using an automated blood grouping machine (PK7300) with monoclonal anti-Mi^a or polyclonal anti-Hil. Glycophorin variants were defined by serology with monoclonal antibodies against Mi^a, Vw, MUT and Mur, and polyclonal antibodies against Hil, MINY and Hop + Nob (KIPP). The glycophorin variants were further confirmed by immunoblotting and Sanger sequencing. Alloanti-Hil and alloanti-MINY in the plasma were screened using GP.Hil RBCs in an antiglobulin test. The specificity of anti-Hil or anti-MINY was assessed using GP.Hil (Hil+MINY+) and GP.JL (HilMINY+) RBCs.

Results: The GP.HF, GP.Mur, GP.Hut, GP.Vw, GP.Kip and GP.Bun frequencies in 1 005 594 individuals were 0·0357%, 0·0256%, 0·0181%, 0·0017%, 0·0009% and 0·0007%, respectively. GP.Hil was found in as four of the 13 546 individuals (0·0295%). Of 137 370 donors, 10 had anti-Hil (0·0073%) and three had anti-MINY (0·0022%).

Conclusions: Glycophorin variants were relatively rare in Japanese individuals, with the major variants being GP.HF (0·0357%), GP.Hil (0·0295%) and GP.Mur (0·0256%). Only one example of anti-MINY was previously reported, but we found three more in this study.

KEYWORDS
glycophorin gene, glycophorin variant, Miltenberger phenotype, MNS blood group

INTRODUCTION

MNS is a highly complex blood group comprising 50 antigens that have been recognized by the International Society of Blood Transfusion [1]. These antigens are carried on two sialic acid-rich glycoproteins, glycophorin A (GPA) and glycophorin B (GPB), or on hybrid molecules composed of portions of GPA and GPB. The glycophorin variants, previously referred to as Miltenberger phenotypes [2], carry a single or multiple unique antigens as follows: Mi^a (MNS007), Vw (MNS009), Mur (MNS010), Hut (MNS019), Hil (MNS020), Hop (MNS026), Nob (MNS027), DANE (MNS032), TSEN (MNS033), MINY (MNS034), MUT (MNS035) and KIPP (MNS048).

Glycophorin A, GPB and GPE are encoded by highly homologous and closely related genes, *GYPA*, *GYPB* and *GYPE*, respectively. *GYPA* is composed of seven exons (A1 to A7), whereas *GYPB* and *GYPE* are composed of six (B1 to B6 and E1 to E6, respectively). B3, E3 and E4 are pseudoexons because of mutation of the splicing donor sites. In addition, E6 has a premature stop codon. However, several glycophorin genes encoding glycophorin variants have a hybrid exon 3 generated by recombination between A3 of *GYPA* and B3 of *GYPB*, resulting in the above unique antigens. The predicted amino acid sequence of glycophorin variants, corresponding to part of exons 2 to 4 of the glycophorin genes and the deduced epitopes for the specific antibodies [3–6] are shown in Fig. 1.

Anti-Mi^a reacts with GP.Vw, GP.Hut, GP.Mur, GP.Hop, GP.Bun, GP.HF and GP.Kip RBCs [7,8], but not with GP.Hil which expresses Hil and MINY antigens on RBCs (Table 1). GP.Mur is the most common glycophorin variants in South-East Asia, with a mean frequency of 7·3% in Taiwan [9], 8·0% or more in Thailand [10,11], 6·3% in Hong Kong [12], 7·6% in Filipino [13], 6% in Vietnam (Hanoi) [14] and 2·8% in Malaysia [15]. In contrast, in our early study, only 0·006% (1 in 16 000) GP.Mur individual was detected in Japanese [16]. Although the approximate frequency of GP.Mur is only 0·01% in Caucasians [17], it is relatively higher in the Australian population (0·1%, 5 in 5098) [18]. However, due to the shortage of anti-Hil, no frequency study on GP.Hil has been reported. We therefore investigated the distribution of glycophorin variants, including GP.Hil, and the distribution of alloantibodies against Hil and MINY antigens among Japanese blood donors.

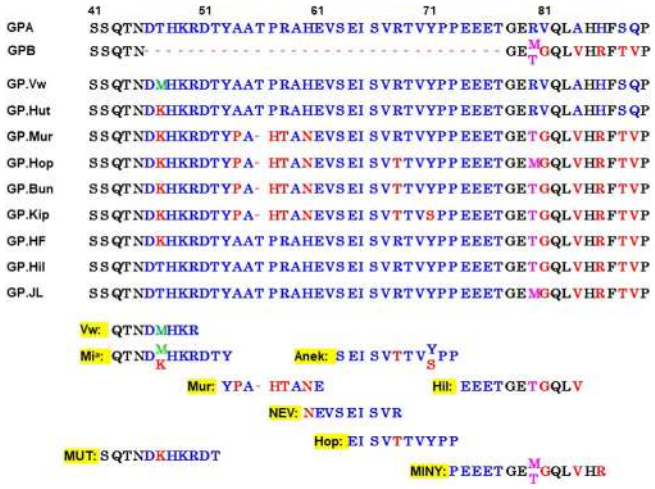


FIGURE 1 Alignment of amino acid residues 41–90 of the GPA, GPB and glycophorin variants. Predicted epitopes for glycophorin variant antigens are shown below [Colour figure can be viewed at wileyonlinelibrary.com]

MATERIALS AND METHODS

Blood samples and serology

This study was approved by the ethics committee of the Japanese Red Cross Society (#2018-003). Blood samples from 1 005 594 blood donors at the Kanto-Koshinetsu Block Blood Center were subjected to Mi(a+) screening using an automated blood grouping machine (PK7300; Beckman Coulter, Koto-ku, Tokyo, Japan) with murine monoclonal anti-Mi^a (CBC-172) [19]. Another set of 13 546 blood samples was subjected to Hil+ screening by PK7300 using alloanti-Hil obtained from blood donors. Glycophorin variants were defined by standard tube tests using in-house monoclonal antibodies against Mi^a (CBC-172), Vw (CBC-430), MUT (CBC-412), Mur (HIRO-138; 3-32/64-D6 [19]) and NEV (CBC-181; 3-29/64-2A3 [19]), and polyclonal antibodies to Hil, MINY, Hop and Hop + Nob (KIPP) obtained from blood donors. Plasma samples from 137 370 blood donors were screened by the polyethylene glycol antiglobulin test with GP.Hil RBCs to detect anti-Hil and anti-MINY. The specificity of anti-Hil and anti-MINY was assessed using five GP.Hil (Hil+ MINY+) RBC samples and one GP.JL (Hil MINY+) RBC sample collected from blood donors.

Immunoblotting

Immunoblot analysis of RBC membrane proteins was performed according to the method described previously [20] using monoclonal anti-M (CBC-3; 2B-23/4C9) [21] or anti-N (CBC-14, in-house).

TABLE 1 Serological definition of glycophorin variants and associated antigens

Phenotype GP (Mi)	Anti-Mia	Vw	MUT	Mur	Hil	MINY	Hop	KIPP	NEV
GP.Vw (Mi.I)	+	+	−	−	−	−	−	−	−
GP.Hut (Mi.II)	+	−	+	−	−	−	−	−	−
Gp.Mur (Mi.III)	+	−	+	+	+	+	−	−	+
GP.Hop (Mi.IV)	+	−	+	+	−	+	+	NT	NT
GP.Bun (Mi.VI)	+	−	+	+	+	+	+	+	−
GP.Kip	+	−	+	+	+	+	−	+	NT
GP.HF (Mi.X)	+	−	+	−	+	+	−	−	−
GP.Hil (Mi.V)	−	−	−	−	+	+	−	−	−
GP.JL (Mi.XI)	−	−	−	−	−	+	−	−	−

Glycophorin gene analysis

Genomic DNA was extracted from whole blood using a DNA blood mini kit (QIAamp, Qiagen, Chuo-ku, Tokyo, Japan). Exons 2 to 4 of the *GYP* genes were amplified by PCRs 1-3 and then analysed by Sanger sequencing using the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) and Genetic Analyzer (model 3500xl, Applied Biosystems). For detailed materials and methods, see Appendix S1, which is available as supporting information in the online version of this paper.

RESULTS

Prevalence of the glycophorin variants

Red Blood Cell samples from 831 out of the 1 ,005,594 individuals (0.0826%) were positive for anti-Mi^a (Table 2). Further serological tests revealed that 359 samples were type GP.HF (0.0357%), 257 samples were GP.Mur (0.0256%), 182 samples were GP.Hut (0.0181%), 17 samples were GP.Vw (0.0017%), 9 samples were GP.Kip (0.0009%), and 7 samples were GP.Bun (0.0007%). In addition, four RBC samples with GP.Hil were identified from another set of 13 546 RBC samples (0.0295%). Although data are not shown, the glycophorin variants were further confirmed by Sanger sequencing. Of note, in the GP.Hil individuals (proband-1 to -4), proband-1 was M + N + S−s+, proband-2 was M−N + S−s+ and proband-3 and -4 were M + N−S−s+. Proband-1 and -2 had the *GYP(A^M-B)*Hil* allele, whereas proband-3 and -4 had the *GYP(A^N-B)*Hil* allele. Furthermore, three different *A-B* breakpoints in intron 3 of the *GYP*Hil* alleles were identified (Figure S1).

TABLE 2 Glycophorin variants and frequencies in Japanese individuals

Phenotype no. GP (Mi)	Total detected	No. tested	Frequency %
GP.HF (Mi.X)	359	1,005,594	0.0357
GP.Mur (Mi.III)	257	1,005,594	0.0256
Gp.Hut (Mi.II)	182	1,005,594	0.0181
GP.Vw (Mi.I)	17	1,005,594	0.0017
GP.Kip	9	1,005,594	0.0009
GP.Bun (Mi.VI)	7	1,005,594	0.0007
GP.Hil (Mi.V)	4	13,546	0.0295

Immunoblot analysis

Immunoblots of GP.Hil RBC membranes with anti-M and anti-N to detect GP(A^M-B).Hil and GP(A^N-B).Hil, respectively, are shown in Fig. 2a,b. Four abnormal bands were observed in proband-1 and proband-2 with anti-M compared with M+N or M+N+ RBCs, which correspond to the monomeric forms of GP(A^M-B).Hil, heterodimer of GP(A^M-B).Hil with GPB, dimer of GP(A^M-B).Hil and heterodimer of GP(A^M-B).Hil with GPA. Similar abnormal bands of GP(A^N-B).Hil were observed in proband-3 and proband-4 with anti-N. Of note, *GYP(A-B)*, encoding the GP(A-B) hybrid molecule, which is hypothesized to be generated by recombination between *GYPA* and *GYPB*, results in the loss of normal *GYPA* and *GYPB* [9]. Therefore, individuals with GP(A-B) hybrid molecule may have a single normal GPA and GPB. However, proband-1 had both GPA.M and GPA.N. In addition, when we analysed reticulocyte mRNA obtained from proband-1, we identified both *GYPA*M* and *GYPA*N* transcripts (data not shown).

Frequencies of anti-Hil and anti-MINY

Among the 137 370 plasma samples, 13 (0·0095%) were positive for GP.Hil RBCs by antiglobulin tests (Table 3). Three of the 13 plasma samples were also positive for GP.JL RBCs, and thus, the frequencies

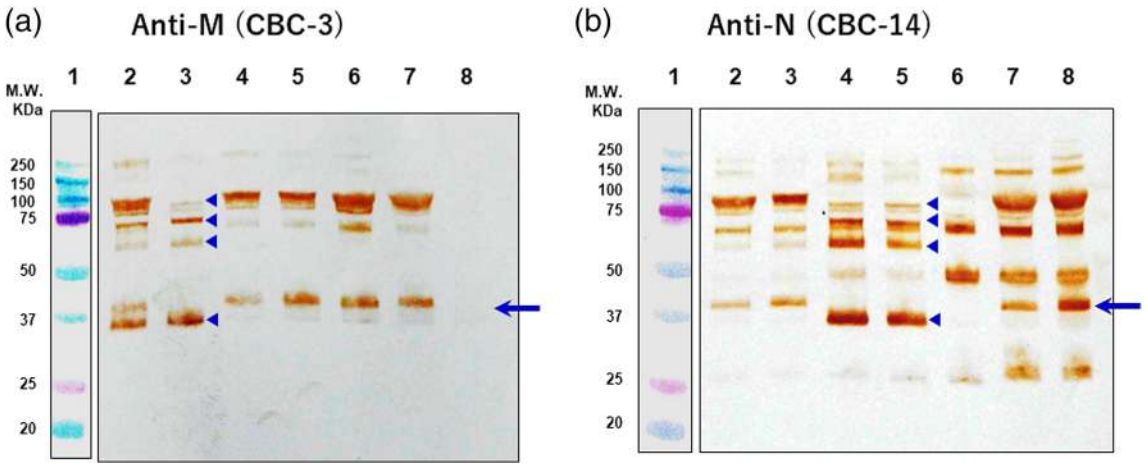


FIGURE 2 Immunoblot analysis of the RBC membranes obtained from GP.Hil+ probands. Lane 1; molecular size marker, lane 2–5; GP.Hil from proband-1 to proband-4, lane 6; MM homozygous control, lane 7; MN heterozygous control, lane 8; NN homozygous control. (a) Stained with anti-M (CBC-3). (b) Stained with anti-N (CBC-14). Triangles indicate abnormal bands and arrows indicate monomeric GPA [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Frequencies of anti-Hil and anti-MINY in Japanese individuals

Antibody in plasma	No. detected	No. tested	Frequency (%)
Anti-Hil	10 (6 Males, 4 females)	137 370	0.0073
Anti-MINY	3 (2 Males, 1 female)	137 370	0.0022

of anti-Hil and anti-MINY in Japanese individuals were 0·0073% and 0·0022%, respectively.

DISCUSSION

The complexity of MNS blood group antigens is due not only to single-nucleotide polymorphism of *GYPA* and *GYPB* genes, but also to recombination among *GYPA*, *GYPB* and *GYPE*. The hybrid glycophorin genes encode glycophorin variants, which express abnormal antigens, such as Mi^a, Vw, MUT, Mur, Hil, MINY and Hop, and some of these antigens can induce clinically significant antibodies in patients or pregnant women by immunization [17,22-27,30]. It is therefore important to systematically identify the glycophorin variants and clarify their frequencies in populations [18,28].

In this study, we screened glycophorin variants using monoclonal anti-Mi^a or polyclonal anti-Hil, and further classified them by serology, immunochemistry and molecular genetic analysis. Although glycophorin variants were rare in Japanese, we identified six different Mi^a-positive hybrid glycophorins, GP.HF (0·0357%), GP.Mur (0·0256%), GP.Hut (0·0181%), GP.Vw (0·0017%), GP.Kip (0·0009%) and GP.Bun (0·0007%), among the 1 005 594 Japanese individuals, but GP.Hop was not detected. GP.HF is a unique GP(B-A-B) variant that has not been reported in non-Japanese populations. In contrast, GP.Mur is relatively common in Asian populations [9–15] but rare in Japanese and northern Chinese [29]. GP.Hut (Mi.II) and GP.Vw (Mi.I) are found in approximately 0·06% of Caucasian [8] and less frequently in Japanese.

In addition, we identified four GP.Hil individuals (proband-1 to −4) in another set of 13 546 individuals (0·0295%). Immunoblotting and sequence analysis revealed that proband-1 and proband-2 had GP(A^M-B).Hil, whereas proband-3 and proband-4 had GP(A^N-B).Hil. The breakpoints of *GYP*Hil* in intron 3 had varied widely in these samples (Fig. S1), but the breakpoints of two *GYP*Hil* alleles for proband-1 and proband-4 were identical. *GYP*Hil* alleles are hypothesized to be generated by recombination between *GYPA* and *GYPB* [8] (Event 1; Fig. S2), resulting in the loss of normal *GYPA* and *GYPB*. Therefore, individuals with *GYP*Hil* hybrid genes have a single normal *GYPA* and *GYPB* and may have neither M+N+ nor S+s+ types. However, based on immunoblot analysis, proband-1 had both normal GPA.M and GPA.N. When *GYPA* transcripts of proband-1 were analysed by PCR, cloning and sequencing using cDNA synthesized from reticulocyte mRNA, we identified both *GYPA*M* and *GYPA*N* transcripts (data not shown). Therefore, proband-1 may have *GYP*Hil* linked with normal *GYPA*, which was generated by secondary recombination (Event 2; Fig. S2).

A few anti-Hil examples [13,25] and only one anti-MINY example [6] have been reported. These examples were from women with a history of pregnancy and/or transfusion. In this study, we screened anti-Hil and anti-MINY in the plasma of 137 370 healthy blood donors. The frequencies of anti-Hil and anti-MINY were 0·0073% (10 individuals) and 0·0022% (3 individuals), respectively, but six of the 10 individuals with anti-Hil and two of the three individuals with anti-MINY were males who had no history of transfusion. Anti-Hil and anti-MINY were likely naturally occurring antibodies in these individuals, but their clinical significance remains to be investigated.

ACKNOWLEDGEMENTS

We are grateful to all the staff members of the Blood Group Laboratories of Kanto-Koshinetsu Block Blood Center.

S.K., A.M., T.C. and S.O. performed serological tests; S.Y. performed immunoblotting; K.I. and K.O. analysed DNA and mRNA data; K.O. and M.U. wrote the paper, which was edited by N.H.T. and M.S.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Kenichi Ogasawara https://orcid.org/0000-0001-7675-7227
Nelson-Hirokazu Tsuno https://orcid.org/0000-0002-7619-6500

REFERENCES

1. The International Society of Blood Transfusion. Red cell immunogenetics and Blood Group Terminology Working Party. http://www.isbtweb.org/fileadmin/user-upload/Working-parties/WP-on-Red-Cell-Immunogenetics-and/Table-of-blood-group-antigens-within-systems-v8.1-181111.pdf. Accessed 24 June 2021.
2. Tippet P, Reid ME, Poole J, Green CA, Daniels GL, Anstee DJ. The Miltenberger subsystems: is it obsolescent? Transfus Med Rev. 1992; 6:170–82.
3. Dahr W. Miltenberger subsystem of the MNSs blood group system. Review and outlook. Vox Sang. 1992;62:129–35.
4. Johe KK, Vengelen-Tyler V, Leger R, Blumenfeld OO. Synthetic peptides homologous to human glycophorins of the Miltenberger complex variants of MNSs blood group system specify the epitopes for Hil, SJL, Hop, and Mur antisera. Blood. 1991;78:2456–61.
5. Heathcote D, Carroll T, Wang JJ, Flower R, Rodionov I, Tuzikov A, et al. Novel antibody screening cells, MUT+Mur kodecytes, created by attaching peptides onto red blood cells. Transfusion. 2010;50: 635–41.
6. Reid ME, Poole J, Green C, Neill G, Banks J. MINY: a novel MNS-related blood group antigen. Vox Sang. 1992;63:129–32.
7. Lopez GH, Wei L, Ji Y, Condon JA, Luo G, Hyland CA, et al. GYP*Kip, a novel GYP(B-A-B) hybrid allele, encoding the MNS48 (KIPP) antigen. Transfusion. 2016;56:539–41.
8. Daniels Geoff. MNS Blood Group System. Human Blood Groups. 3rd ed. Oxford, UK, Wiley-Blackwell; 2013:96–161.
9. Broadberry RE, Lin M. The incidence and significance of anti-"Mi^a" in Taiwan. Transfusion. 1994;34:349–52.
10. Palacajornsuk P, Nathalang O, Tantimavanich S, Bejrachandra S, Reid ME. Detection of MNS hybrid molecules in the Thai population using PCR-SSP technique. Transfus Med. 2007;17: 169–74.

11. Jongruamklang P, Gassner C, Meyer S, Kummasook A, Darlison M, Boonlum C, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of 36 blood group alleles among 396 Thai samples reveals region-specific variants. Transfusion. 2018; 58:1752–62.
12. Poole J, King MJ, Mak KH, Liew YW, Leong S, Chua KM. The MiIII phenotype among Chinese donors in Hong Kong: immunochemical and serological studies. Transfus Med. 1991;1:169–75.
13. Hsu K, Lin YC, Chao HP, Lee TY, Lin M, Chan YS. Assessing the frequencies of GP.Mur (Mi.III) in several Southeast Asian populations by PCR typing. Transfus Apher Sci. 2013;49:370–1.
14. Huynh NT, Ford DS, Duyen TT, Huong MT. Jk and Mi.III phenotype frequencies in north Vietnam. Immunohematology. 2003;19:57–8..
15. Prathiba R, Lopez CG, Usin FM. The prevalence of GPMur and anti-"Mi^{a}" in a tertiary hospital in Peninsula Malaysia. Malaysian J Pathol. 2002;24:95–8.
16. Uchikawa M, Tsuneyama H, Akaza T, Tadokoro K, Juji T. Miltenberger antigens in Japanese. Book of Abstracts 6th Regional Congress of International Society of Blood Transfusion, Western Pacific Region; 1995:100. https://www.isbtweb.org/about-isbt/history?tx_isbtarchive_history%5B%40widget_0%5D%5BcurrentPage%5D=10&cHash=52ef7e8b0c1ab02887e40efe0738dc06
17. Race RR, Sanger R. The MNSs Blood Groups. Blood Groups in Man. 6–138. Oxford, Blackwell Scientific Publications LTD; 1975:92.
18. Lopez GH, Wlison B, Turner RM, Millard GM, Fraser NS, Roots NM, et al. Frequency of Mi^{a} (MNS7) and classification of Mi^{a}-positive hybrid glycophorins in an Australian blood donor population. Transfus Med Hemother. 2020;47:279–86.
19. Reid ME, Lisowska E, Blanchard D. Section 3: epitope determination of monoclonal antibodies to glycophorin A and glycophorin B. Coordinator's report. Antibodies to antigens located on glycophorins and band 3. Transfus Clin Biol. 2002;9:63–72.
20. Tsuneyama H, Isa K, Watanabe-Okochi N, Ogasawara K, Uchikawa M, Satake M. An unusual variant glycophorin expressing protease-resistant M antigen encoded by the *GYPB-E(2–4)-B* hybrid gene. Vox Sang. 2020;115:579–85.
21. Rasamoelisolo M, Rahuel C, Loirat MJ, Willem C, Carron JP, Blanchard D. Immunochemical characterization of monoclonal antibodies directed to glycophorins A and/or B. Transfus Clin Biol. 1997;1:91–6.
22. Lyndall M. Intravascular hemolytic transfusion reaction due to anti-Vw+Mia with fatal outcome. Vox Sang. 1981;40:105–8.
23. Taylor AM, Knighton GJ. A case of severe hemolytic disease of the newborn due to anti-Verweyst (Vw). Transfusion. 1982;22:165–6.
24. Lin M, Broadberry RE. An intravascular hemolytic transfusion reaction due to anti-'Mi^{a}' in Taiwan. Vox Sang. 1994;67:320.
25. Lin CK, Mak KH, Cheng G, Lao TTH, Tang MHY, Yuen CMY. Serologic characteristics and clinical significance of Miltenberger antibodies among Chinese patients in Hong Kong. Vox Sang. 1998;74: 59–60.
26. van den Bos AG, Steiner K. Haemolytic disease of the newborn caused by anti-MUT (MNS 35). Vox Sang. 2004;87:208–9.
27. Mallari RA, Chan A, Powers RJ, Pandipati S, Bensing KM, Biese D, et al. Fetal inheritance of GP*Mur causing severe HDFN in an unrecognized case of maternal alloimmunization. Transfusion. 2020; 60:870–4.
28. Lomas-Francis C. Miltenberger phenotypes are glycophorin variants: a review. ISBT Sci Ser. 2011;6:296–301.
29. Broadberry RE, Lin M. The distribution of the MiIII (GP.Mur) phenotype among the population of Taiwan. Transfus Med. 1996;6:145–8.
30. Ellisor SS, Zelski D, Sugawara E, Dean WD, Bradburn S. A second example of anti-Hil. Transfusion. 1982;22:402.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Kaito S, Suzuki Y, Masuno A, Isa K, Toyoda C, Onodera T, et al. Frequencies of glycophorin variants and alloantibodies against Hil and MINY antigens in Japanese. Vox Sang. 2021;1–5.

Received: 22 July 2020 | Revised: 16 April 2021 | Accepted: 2 May 2021

DOI: 10.1111/vox.13133

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The efficacy of ethnic specific blood groups genotyping for routine donor investigation and rare donor identification in Taiwan

Meng-Hua Yang[1,2] | Jen-Wei Chen[1] | Sheng-Tang Wei[1] | Sheng-Mou Hou[1,3] | Yann-Jang Chen[2,4,5,6]

[1]Head Office, Taiwan Blood Services Foundation, Taipei, Taiwan

[2]Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

[3]Department of Orthopedic Surgery, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan

[4]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan

[5]Department of Pediatrics, Taipei Veteran General Hospital, Taipei, Taiwan

[6]Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

Correspondence
Yann-Jang Chen, Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan.
Email: yjchen@ym.edu.tw

Abstract

Background: Large-scale single nucleotide variation (SNV)-based blood group genotyping assays have been made available for over a decade. Due to differences in ethnic groups, there is much diversity in clinically important blood group antigens and genetic variants. Here, we developed a robust matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-based blood group genotyping method on MassARRAY system.

Study Design and Methods: A total of 1428 donors were enrolled into three groups: (a) reagent red cell donors; (b) rare donor or common antigen-negative donors; and (c) group O, R_1R_1/R_2R_2 donors. Forty-two SNVs were designed for determining nine blood groups, with X/Y chromosome in two multiplex reactions, on MassARRAY 96-well format system. Further targeted sequence analyses were performed by Sanger sequencing.

Results: WHO reference reagent (NIBSC code: 11/214) was tested for concordance with the provided genotype results. Among the donors, concordance rate was over 99%. Alleles of important phenotypes such as Mi(a+), Di(a+), and Asian-type DEL and alleles of rare blood groups such as Fy(a−), Jk(a−b−) and s− were screened. Three types of discrepancies were found. Serologically, the 'N' antigen was expressed on genetically MM with GYP*Mur red blood cells and caused genuine discrepancies (9.5%). Genetically, allele dropout (ADO) was caused by rare SNV in the primer for Ss genotype (2.1%) and partial insertion of RHD genes (0.9%) led to difficulties in predicting phenotypes.

Conclusion: Hemo panel module and MassARRAY System in 96-well format showed good performance in terms of large-scale blood group genotyping and phenotype predictions. Implementation of this method is effective for routine blood group genotype screening of donors.

KEYWORDS
blood group antigen, blood group genotyping, ethnic specific blood group, glycophorin hybrids, rare donor identification

ABBREVIATIONS: ADO, allele dropout; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

INTRODUCTION

Matching blood donors to recipients is basic to transfusion safety and is the first example of 'precision medicine' [1, 2]. For patients who need long-term red cell transfusion, such as those with sickle cell disease or β-thalassemia, who are at risk of alloimmunization, suitable RBC units can be difficult to obtain [3]. For patients with antibodies against high frequency antigens or multiple antigens, it can be challenging to source antigen-negative red cell units. The National Institutes of Health (NIH) of the United States established a database of 2700 blood donors with 20 red cell antigen phenotypes in the 1960's [4]. The American Rare Donor Program (ARDP) and its database, REGGI formed in 1998, has phenotype information on more than 59,000 active rare donors submitted till 2014 [5]. In Japan, routine screening tests are conducted using 11 monoclonal antibodies (C, c, E, e, Fy^b, Jk^a, Jk^b, Le^a, M, S and Di^a) to prepare antigen-negative blood for patients with clinically significant alloantibodies [6]. In Italy, serotyping of donors is also a part of rare donor programmes [7]. Due to the lack of some commercial antisera and detection methods, not all clinically relevant blood groups are routinely screened and identified.

Because of the emergence of molecular technologies and research into human genomics, the rapid and accurate testing of known single nucleotide variation (SNV) loci can predict blood groups and screen large populations [8]. Different methods have been reported, such as DNA array [9], bead array [10, 11], kompetitive allele-specific polymerase chain reaction [12], MassARRAY, [13, 14] and massively parallel sequencing (MPS) technology (also called next-generation sequencing) [15, 16].

Extended matching for selected antigens lowered the alloimmunization rate in RBC transfusion [17]. In Taiwan, serological screening of C, c, E, e, Jk^a, Jk^b and M is performed for selected donors by annual projects. It helps to provide antigen-negative RBC units for transfusion. Besides, the selections of reagent red cell panels for alloantibodies screening and identification also need results of more blood group antigens. For other clinically relevant phenotypes, such as Mi(a+), Di(a+); the data are limited due to the unavailability of commercial antisera for screening [18]. Genotyping of ethnic-specific blood group alleles such as *RHD*DEL1*, *JK*02N* and *GYP*Mur* have been assayed separately for limited cases to clarify the genetic basis [19–21]. Results of *RHD*DEL1* can avoid to provide DEL blood units to recipients developing allo-anti-D. Identifying *JK*02N* alleles benefits to select true homozygous *JK*A/JK*A* and *JK*B/JK*B* donors for reagent red cell panel. Genotyping of *GYP*Mur* is a major assay of confirming the glycophorin hybrids when lacking MoAb of specific antigens. It was also reported that GP.Mur phenotype is associated with an elevated expression of 'N' on GPB. The molecular origin of 'N' antigen is on N-terminal pentapeptides of GPB and is similar to the N antigen on GPA [22]. In clinical blood banking practice in Taiwan, some patients with M + N- and GP.Mur phenotype would be weak agglutination with undiluted anti-N sera, which was also reported in Thai people [14] and this phenomenon can also be clarified by genotyping. To utilize the benefits of SNV-based technologies and evaluate the feasibility of large-scale donor screening for multiple blood groups, we performed a pilot study of three platforms on 133 Taiwanese blood donors. These included two commercially available platforms (inno-train RBC-FluoGene vERYfy, and Grifols ID CORE XT) and one in-house testing panel (Hemo Panel version I) with MassARRAY platform.

In this study, we investigated blood group genotyping on a large scale via a MassARRAY platform using custom-designed panel. We modified primer sets to improve the reaction efficiency for Hemo Panel version II (Table 1 and Table S1). On Hemo Panel version II, SNVs in *ABO* gene and SNV in *AMEL* of XY chromosome, and two polymorphic SNVs rs136337 and rs2010235 all helped to compare the results with donors' basic information to reduce possible sample mix-ups. Taking the criteria from the American Rare Donor programme as a Reference [5], we enrolled donors into three categories: (a) reagent red cell donors; (b) Common antigen-negative donors; and (c) Group O, R_1R_1 or R_2R_2 donors. This assay is a robust and reliable method for clinically important blood group genotyping and well suited for use in routine laboratories.

MATERIALS AND METHODS

WHO reference reagent 11/214

To verify the in-house blood group genotyping set, Hemo Panel version II, the WHO International Reference Reagent for blood group genotyping (11/214) supplied by the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK) was tested [23]. This panel contained four types of genomic DNA (10/232, 10/236, 10/238, 10/234). The results from Hemo Panel version II were compared with the listed genotypes as per the instruction.

Donor recruitment and sample preparation

We recruited 1428 blood donors into three categories for blood group genotyping. They included (a) 219 reagent red cell donors, among them those with ethnic-specific blood groups Mi(a+) and Di(a+); (b) 244 rare donors or common antigen-negative donors such as D-negative, DEL, RzRz, Jk(a−b−), s−, Fy(a−), and K_o; and (c) 965 group O donors with regular R_1R_1 and R_2R_2. DNA from all donors was extracted from buffy coat samples using automated LabTurbo 48 Compact System and LabTurbo nucleic acid purification kit (Taigen Bioscience, Taipei, Taiwan).

Serological blood grouping

Historical serological blood groups of selected donors were from the donors' database. Most Rh (D, C, c, E, e) and Kidd (Jk^a, Jk^b) antigen results were by the PK7300 Automated Microplate System (BECKMAN

COULTER, USA) or column agglutination technology on ORTHO VISION Max analyser (Ortho-Clinical Diagnostics, UK). Detailed RhD antigen typing was implemented following weak D test, based on the manufacturer's instructions. Further adsorption and elution test for D antigen was performed as needed [19].

Blood group genotyping on MassARRAY platform: Hemo Panel

All samples were genotyped on MALDI-TOF MS platform (MassARRAY, Agena Bioscience GmbH) using in-house designed panels referred as Hemo Panel version I and II, respectively. Forty-two SNVs were designed in Hemo Panel version II (Table 1, Table S1). Common antigen-determining SNVs were selected in eight blood group systems, including MNS, RH, LU, KEL, FY, JK, DI and DO. Next, SNVs of ethnic-specific antigens and weak/null phenotypes were selected, such as Mi^a, Di^a, DEL, Jk(a$+^{weak}$) and Jk(a−b−). For basic identification of donors, SNVs for ABO blood group system, *AMEL* gene on X/Y chromosome, and two polymorphic SNVs (rs136337, rs2010235) were included.

MALDI-TOF MS analysis

SNV genotyping was performed on Agena MassARRAY platform with iPLEX gold chemistry (Agena, San Diego, California) following the manufacturer's instructions. Analysis of SNVs was carried out with TYPER 4.0 software.

Predicted phenotype analysis

We exported the data files with TYPER 4.0 software and converted to predicted phenotypes or antigens by computer software (Excel 2016, Microsoft Corp.). Primers of *RHD* and *RHCE* were designed to co-amplify SNV-containing region of both genes, and unextended primers (UEPs) detected the same positions SNVs from both genes. Allele peak ratios were used to predict the number of copies of *RHD* compared to *RHCE* [24, 25]. For analysing *RHD* genes, each plate should contain known *DD*, *Dd* and *dd* as controls. SNVs of c.307 C/T and c.676G/C in *RHCE* resulted in the c/C and e/E antigens, but in *RHD* gene template, they were c.307T and c.676G, respectively. The allelic peak ratios helped to determine calling both SNVs. The cluster plots of SNVs were checked. Ambiguous SNV calling may occur among partial deletion of amplified regions in both genes. If the possible deletions were observed, sequence analyses were needed.

Characterization of GYP(B-A-B) hybrid

Serologically Mi(a+) donors and *GYP*Mur/GYP*Bun* donors were analysed for hybrid alleles. The genotyping protocol of *GYP(B-A-B)* hybrid has been previously described [25].

Sanger sequencing

To confirm the *Ss* genotypes of three discrepant donors, we amplified *Ss* gene with primer set S-1: TCTTTGTCTTTACAATTTCGTGTGA and S-2: CATGAATACGTGTTGGGTCA.

For the detailed sequence analysis of *GYPA* and *GYPB* hybrids, partial exon 3 and intron 3 were amplified by primer set GYPAB-E3 Primer1: ACGTTGGATGGTGCCCTTTCTCAACTTCTC and GYPAB-E3 Primer2: ACGTTGGATGCAGTTAATAGTTGTGGGTGC.

Sanger sequencing in both directions was performed on ABI 3730xl DNA analyser (Applied Biosystems, Inc., Foster City, California).

RESULTS

Pilot study

Among the data obtained from Hemo Panel version I with MassARRAY platform, the genotype–phenotype data of this Hemo Panel version I demonstrated 100% concordance with predicted phenotypes, including Kp^a/Kp^b, Lu^a/Lu^b antigens, with a sample size of 133. For the commercial platform, Grifols ID CORE XT, one SNV (rs1253504488) in the primer site caused allele dropout (ADO) and a discrepancy on Kidd system. In dbSNP, the frequency of rs1253504488 in Asian population is 100% C allele. But it was a C-to-A variation in our sample (Figure S2). And in inno-train RBC-FluoGene vERYfy (v1.3.2.0), no null SNVs of Kidd system were included in this assay and caused discrepancies when *JK*02N.01* was showed in one donor. The pilot study also showed that the SNVs in commercial genotyping panels might be not sufficient for Asian ethnic donor screening.

WHO reference reagent

Genomic DNA panel (NIBSC code: 11/214) for blood group genotyping was prepared following the provided instructions [23]. For four DNA samples (NIBSC code: 10/232, 10/236, 10/238, 10/234), genotyping was performed using Hemo Panel version II. All results were in concordance with the genotypes provided in the instructions, except for *RHDΨ*, which was not included in Hemo Panel version II.

Donor samples

A total of 1428 donors were enrolled into three groups: 219 reagent red cell donors, 244 rare or common antigen-negative donors, and 965 group O donors with known R_1R_1/ R_2R_2 phenotypes. Eight hundred and fifty donors from R_1R_1/ R_2R_2 group were tested with Hemo Panel version I assay (Figure S1), while other 578 donors were tested with Hemo Panel version II assay (Table 1).

TABLE 1 SNVs on Hemo Panel, with allele frequencies and predicted antigen frequencies of 965 group O, R_1R_1/R_2R_2 donors

Blood group system	Phenotype	Gene	SNV	Allele	Allele frequency		Predicted phenotype	Frequency
Kidd	Jk^b/Jk^a	*SLC14A1*	c.838A/G	*JK*02/JK*01*	G:0.55	A: 0.45	Jk(a + b−)	21.5%
	Jk(a−b−)		c.342−1G/A c.342−1G/C	*JK*01N.06*, *JK*02N.01*, *JK*02N.02*	G: 0.98	A: 0.02	Jk(a + b+) Jk(a−b+)	48% 30.5%
			c.896G/A	*JK*02N.07*	G: >0.99	A: <0.01		
			c.222C/A	*JK*02N.03*	C: >0.99	A: <0.01		
			c.499A/G	*JK*02N.11*, *JK*02N.12*, *JK*02N.13*	A: 0.78	G: 0.22		
			−103A/G[b]	*Polynesian* Jk^{null}	A: 0.98	G: 0.02		
			−119C/A[b]	*Polynesian* Jk^{null}	C: 0.98	A: 0.02		
	$Jk(a+^w)$ [b]		c.130G/A	*JK*01W.01*	G: 0.63	A: 0.37		
Kell	k/K	*KEL*	578C/T	*KEL*01.01/KEL*02*	C: 1		K−k+	100%
	Kp^b/Kp^a		841C/T	*KEL*02.04/KEL*02.03*	C: 1		Kp(a−b+)	100%
	Js^b/Js^a		1790 T/C	*KEL*02.07/KEL*02.06*	T: 1		Js(a−b+)	100%
	K_0		1546C/T	*KEL*02N.17*	C: 1			
			715G/T	*KEL*02N.24*	G: 0.99	T: 0.01		
			730 delG	*KEL*02N.27*	G: 1			
Duffy	Fy^b/Fy^a	*ACKR1*	125A/G	*FY*02/FY*01*	A: 0.05	G: 0.95	Fy(a+b−)	90%
	Fy(a−b−)		−67T/C	*FY*01N.01*, *FY*02N.01*	T: 1		Fy(a+b+)	10%
	$Fy(a+^w)$, $Fy(b+^w)$		265C/T	*FY*01W*, *FY*02W*	C: 1		Fy(a−b+)	<0.1%
Lutheran	Lu^b/Lu^a	*LU*	230G/A	*LU*02/LU*01*	G: 1		Lu(a−b+)	100%
	Au^b/Au^a		1615G/A	*LU*19/LU*18*	A: 0.88	G: 0.12	Au(a+b−)	77.6%
							Au(a+b+)	20.8%
							Au(a−b+)	1.6%
Diego	Di^b/Di^a	*DI*	2561C/T	*DI*02/DI*01*	C: 0.98	T: 0.02	Di(a−b+)	96.3%
							Di(a+b+)	3.7%
	Wr^b/Wr^a		1972G/A	*Di*04/Di*03*	G: 1		Wr(a−b+)	100%
MNS	M/N	*GYPA*	59C/T	*GYPA*01/GYPA*02*	C: 0.59	T: 0.41	M+N−	31.7%
			72T/G	*GYPA*01/GYPA*02*	G: 0.57	A: 0.43	M+N+	50.3%
							M−N+	18%
	s/S	*GYPB*	143C/T	*GYPB*04/GYPB*03*	C: 0.95	T: 0.05	S+s−	0.5%
							S+s+	8%
							S−s+	91.5%
	Mi^a	*GYP B-A-B*	*GYPA* specific SNV	*GYP*Mur/GYP*Bun*	*GYP B-A-B*: 0.02	*GYPB*:0.98	Mi^b	4.3%
Rh	-	*RHD/CE*	Promotor region	*RHD/RHCE*				
	D	*RHD*	Intron 1	*RHD/RHCE*				
			Exon 3	*RHD/RHCE*				
			Exon 4	*RHD/RHCE*				
	DEL		1227G/A	*RHD*01EL.01*	G: 0.99	A: 0.01		
	c/C	*RHCE*	307C/T	*RHCE*c/RHCE*C*				
	e/E		676G/C	*RHCE*e/RHCE*E*				
Dombrock	Do^a/Do^b	*DO*	793A/G	*DO*01/DO*02*	A: 0.1	G: 0.9	Do(a+b−)	1.2%
							Do(a+b+)	18.1%
							Do(a−b+)	80.7%

(Continues)

TABLE 1 (Continued)

Blood group system	Phenotype	Gene	SNV	Allele	Allele frequency	Predicted phenotype	Frequency
ABO [b]	O	*ABO*	261delG	*ABO*O01*			
	B		703C/T	*ABO*B*			
	B		803C/G	*ABO*B*			
	A		467C/T	*ABO*A*			
	A_{el}		374+5G/A	*ABO*AEL.04*			
	B_3		155+5G/A	*ABO*B3.03*			
XY chromosome[b]	X/Y	*AMEL*	DEL/GAT				
Q150-rs136337[b]		*OSBP2*	C/A				
Q150-rs2010235[b]		*NOSTRIN*	C/A				

Abbreviation: SNV, single nucleotide.
[a]In intron 1.
[b]Added in Hemo Panel Version II.
[Correction added on 22 July 2021 after first online publication: In table 1, some footnotes and superscripts have been corrected in this version.]

Reagent red cell donors: Comparisons of serology and genotyping results

Among the 3339 known antigen results from 219 reagent red cell donors on ABO, RH, MNS, Mi[a], Kidd, Kell, Duffy and Diego systems (Table 2), 3314 results showed concordance and 25 results showed discrepancy (concordance rate: 99.25%). Genotype and phenotype results of Kidd, Kell, Duffy, Diego and ABO systems showed full concordance. For Kidd system, three Jk(a + b−) donors and one Jk(a+) donor had *JK*02N* alleles. In addition, three of these four donors were detected *JK*01W.01/JK*02N.01* alleles and all had common Jk(a+) antigen by routine tube methods. For Duffy system, two donors were detected heterozygous for SNV c.265 C>T of weak allele, both were common Fy(a + b+) phenotype. One *FY*02* homozygote and one *DI*01* homozygote donors were first detected by genotyping and were confirmed rare Fy(a−) and Di(a + b−), respectively, on recent donations.

Discrepancies were found on MNS and Rh systems. For the MNS system, 15M+N+ donors had *MM* genotype. On further analyses of the N antigen and Mi[a] antigen, all 15 donors had weakly positive results to anti-N reagents and all had Mi[a] antigen. This indicated that the N+ results were from the expression of enhanced 'N' antigen in hybrid GP(B-A-B) glycophorins [14, 26]. Seventy-four of 75 Mi(a+) donors showed *GYP*Mur* alleles, with confirmed hemi- or homozygous *GYP*Mur* alleles on discrimination assay [25] and Sanger sequencing. One Mi(a+) donor without *GYP*Mur* allele was confirmed to possess *GYP*Hut*.

For the Rh system, all 219 donors were RhD-positive on routine serologic screening with Beckman Coulter PK System. Fifteen donors showed *Dd* haplotypes, one donor showed *RHD*01EL.01* homozygote, and one donor showed partial deletion of *RHD* gene. For the RhCE system, only 2 R_2R_2(ccEE) donors showed the higher *RHD* peak ratios in exon 4 by comparing to controls. For *RHCE*, the ratios of c.307 were nearly 3T: 1C, and c.676 were nearly 1C:1.2G. These caused indeterminate genotypes of *RHCE* gene. This may refer to a hybrid *RHD/RHCE* allele. The zygosity of *RHD* and *RHCE* needed further analysis.

Rare donors or common antigen-negative donors: Detection of null and weak alleles in population

We collected 244 common antigen-negative and weak-antigen expressing donors including those with RhD-negative, Weak D, DEL, Fy(a−b+), S + s−, and Jk(a−b−) and the frequencies in Taiwanese blood donors were listed (Table 3, Table S2 [27]).

RhD-negative, weak D, DEL phenotypes

RhD-negative donors were confirmed by weak D test with monoclonal blend anti-D and anti-human IgG following the manufacturer's instructions. Among 128 RhD-negative donors, 98 (76.6%) showed complete deletion of *RHD* gene, while another 30 donors presented *RHD* variants or *RHD*01EL.01*. Asian-type *DEL* allele (*RHD*01EL.01*) was also detected in two D-negative donors. Twenty-one donors had one copy of *RHD*01EL.01* and two were homozygous for *RHD*01EL.01* allele. Among 28 donors of weak D and DEL, the majority possessed *RHD*01EL.01* allele. Seven donors had one or two copies of *RHD* gene.

R_ZR_Z (DCCEE)

Two R_ZR_Z donors in this study, one was homozygous for *DCE* allele and the other had *DCE* allele and partial deletion of *RHCE* allele.

Fy(a−b+) phenotype

All 29 Fy(a−b+) donors were homozygous for *FY*02* alleles, including one null allele (*FY*02N.01*) and one weak allele (*FY*02W*). No null alleles of *FY*01* were detected.

TABLE 2 Reagent red cell donors[a]

Phenotypes/antigens[a]		Number of donor predicted antigens/genotypes		
		Concordances	Discrepancies	Weak or null allele detected
Kidd ($n = 198$)	Jk(a+b+)	88		
	Jk(a−b+)	57		*JK*02N.01* *JK*02N.07*
	Jk(a+b−)	45[b]		*JK*01W.01* *JK*02N.01*
	Jk(a−b−)	1[c]		*JK*02N.01*
	Jk(a+)	5[d]		*JK*01W.01*
	Jk(a−)	1		
	Jk(b−)	1		
Kell ($n = 122$)	K−k+	122		
Duffy ($n = 175$)	Fy(a+b−)	89		
	Fy(a+b+)	53		*FY*02W* or *FY*01W*[e]
	Fy(a+)	1		
	Fy(b+)	8		
	Fy(b−)	24		
MNS	M+N+	75	15[f] (gynotype:*MM*)	
	M+N−	46		
	M−N+	21		
	M+	24		
	M−	23		
	N+	2		
	N−	1		
	S+s−	1		
	S+s+	42	3[g] (genotype: *SS*)	
	S−s+	93		
	S+	10		
	S−	36		
	Mi^a+	73	1[h]	
	Mi^a−	145		
Rh	D	218	1[i]	*RHD*01N.01* *RHD*01EL.01* Partial Deletion of *RHD*[j]
	CCee	111		
	CcEe	44		
	ccEE	40	2[k] (genotype indeterminate)	
	Ccee	6		
	ccEe	4		
	CCEe	1		
	c−E−	3		
	E−e+	1		
	E-	1		

(Continues)

TABLE 2 (Continued)

Phenotypes/antigens[a]		Number of donor predicted antigens/genotypes		
		Concordances	Discrepancies	Weak or null allele detected
Diego ($n = 176$)	Di(a+b+)	24		
	Di(a+)	49		
	Di(a−)	103		
ABO ($n = 219$)	O	177		*ABO*O.01.01*
	B	24		*ABO*O.01.01*
	A	18		*ABO*O.01.01*

[a]Not all reagent red cell donors have the full antigen profiles.
[b]One donor has *JK*01/JK*02N.01* alleles and two donors have *JK*01W.01/JK*02N.01* alleles.
[c]Homozygous for *JK*02N.01*.
[d]One donor with *JK*01W.01/JK*02N.01* alleles was suggested to be excluded from reagent red cell panel group.
[e]SNV c.265C>T was detected.
[f]'N' antigen.
[g]SNV rs145033200 in primer sequence causes ADO.
[h]GP.Hut donor.
[i]Homozygous for *RHD*01EL.01*.
[j]One donor had partial deletion in one *RHD* gene without further characterized.
[k]Partial insertions of *RHD* gene caused difficulties in interpretation. Partial *RHD* gene was not further characterized.
[Correction added on 22 July 2021 after first online publication: In table 2, the sequence of table legends has been corrected in this version.]

Ss phenotypes

Three S+s+ donors from Reagent red cell donors group were of *SS* genotype. Sequencing of this region revealed a rare SNV (rs145033200) in the original PCR primer, which caused ADO [11]. Three donors were confirmed with *Ss* genotype on Sanger sequencing and modified primers (Table S2). All 11S+s− donors from these 244 donors were homozygous for *GYPB*S* allele.

K_0 phenotype

The K_0 donor we previously reported was also enrolled in this study and assigned *KEL*02N.17/KEL*02N.27* allele [28].

Jk(a−b−) phenotype

Among 46 Jk(a−b−) donors, 28 were homozygous for *JK*02N.01*. Heterozygous *JK*02N.01/JK*02N.07* alleles were detected in 13 donors. In addition, *JK*02N.03* allele was detected in two donors.

R_1R_1/R_2R_2 donors

We recruited O, RhD-positive, R_1R_1, and R_2R_2 donors for genotype analysis. A total of 965 such donors were enrolled and the results of *RHD* and *RHCE* are shown in Table 3. Among them, 830 were R_1R_1 and 135 were R_2R_2. Four had *Dd* genotypes (3 R^1r' and 1 R^2r'') and all were phenotype–genotype concordance for RhCE. The allele frequencies of SNVs and predicted phenotype frequencies are listed in Table 1.

JK

Among *JK*01/JK*02* heterozygote donors, 20 of them were detected *JK*02N* SNVs and predicted Jk(a + b−) phenotype. In this group, 115 donors were tested with Hemo Panel version II, 67 of them were detected *JK*01.W01* allele (c.130G>A) [29]. Notably, one donor who was a *JK*02* homozygote showed one c.130G>A change. This rare allele has also been reported in one African American donor [30].

FY

We detected one *FY*02/FY*02* donor and confirmed rare Fy(a−b+) phenotype on next donation.

GP.Mur, DI

There were 41 donors with *GYP*Mur* allele and 36 donors with *DI*01* allele. *GYP*Mur* donors demonstrated confirmed concordant **Mi(a+)** and 14 of 36 donors with *DI*01* allele were confirmed to have Di(a+).

DISCUSSION

Many blood group genotyping platforms have been introduced in blood centres and blood banks, including commercialized kits and in-house panels [8, 11–14, 31]. We enrolled 850 donors in R_1R_1/R_2R_2 group and tested them with Hemo Panel version I assay. For further identification, we added 10 SNVs to Hemo Panel version II. We then finished genotyping of 578 reagent red cell

TABLE 3 Rare donors or common antigen-negative donors/group O, R_1R_1/R_2R_2 donors

Donor groups	Phenotypes	No. of donors	Alleles	No. of donors
Rare donors/common-antigen-negative donors ($n = 244$)	RhD-negative	128	*RHD*01N.01/RHD*01N.01*	98
			*RHD/RHD*01N.01*	4
			RHD Partial deletion	3
			*RHD*01EL.01/RHD*01N.01*[a]	21
			*RHD*01EL.01/RHD*01EL.01*	2
	Weak D and DEL	28	*RHD/RHD*	2
			*RHD/RHD*01N.01*	5
			*RHD*01EL.01/RHD*01N.01*	13
			*RHD*01EL.01/RHD*01EL.01*	8
	R_ZR_Z	2	*DCE/DCE*	1
			DCE/D-- [b]	1
	Fy(a−b+)	28	*FY*02/FY*02*	25
			*FY*02/FY*02N.01*	1
			*FY*02/FY*02W*[c]	2
	Fy(a−b+), S+s−	1	*FY*02/FY*02, GYPB*S/GYPB*S*	1
	S+s−	10	*GYPB*S/GYPB*S*	10
	Kell-null (K_0)	1	*KEL*02N.17/KEL*02N.27*	1
	Jk(a−b−)	46	*JK*02N.01/JK*02N.01*	28
			*JK*02N.01/JK*02N.07*	13
			*JK*02N.01/JK*02N.03*	2
			*JK*02N.07/JK*02N.07*	3
Group O, R_1R_1/R_2R_2 donors ($n = 965$)	R_1R_1 (CCee)	830	*DCe/DCe*	827
			DCe/dCe	3
	R_2R_2 (EEcc)	135	*DcE/DcE*	134
			DcE/dcE	1

[a]Asian type DEL, *RHD*01EL.01 allele* (*RHD* 1227G>A).
[b]*RHCE* partial deletion.
[c]*FY* c.265C>T.

donors, rare donors or common-antigen-negative donors, and R_1R_1/R_2R_2 group O donors. A total of 1428 samples were analysed. There were only 35 single or sporadic no calls out of all SNV results.

In commercially available genotyping kits, null alleles of *JK* are often limited. But null SNVs of *JK* were detected in our donors and were decisive for predicting Kidd phenotypes. *Jk*02N.01*, *JK*02N.03*, *Jk*02N.07* alleles were detected in Taiwan donor population. For c.130G>A initially reported in *JK*01W.01* allele, we detected a *JK*02* homozygous donor with *JK* c.130G>A in one allele, consistent with a previous report [30]. This indicated the c.130G>A can be detected in both *JK*01* and *JK*02* background, which is similar to the report of c.342-1G>A in both *Jk*01N* and *Jk*02N* alleles. These complex factors should be considered when predicting phenotypes based on genotyping.

For Rh system, the primer sets are designed to co-amplify both *RHD* and *RHCE* genes, and UEPs detect the specific SNVs of both genes. If copy number variation or hybrid alleles are presented, the allele peak ratios of SNVs in *RHD* and *RHCE* genes were used to interpret the semi-quantitative of genotyping data [24, 25]. In this study, one R_ZR_Z donor showed decreased ratios of *RHCE* SNVs in exon 3 and exon 4, which indicated the possible deletion of one copy of *RHCE* gene (Table 3). Among common donors with two copies of *RHD* and *RHCE* genes, this assay had high concordance in R_1R_1 and R_2R_2 donors. Currently, the adsorption and elution test was not fully introduced into the routine D antigen testing. The *RHD*01EL.01* homozygous *DD* donor out of 219 RhD-positive donors (Table 2) was determined RhD positive by automation equipment. It may be because of D antigen detected using column method [32], which was not stated in the database. It needs further investigations for *RHD*01EL.01* in common RhD-positive donors. The incorporation of *RHD*01EL.01* provided important data for predicting RhD phenotypes.

There were 15 discrepancies in terms of *MM* genotypes and MN phenotypes. All were of weak N antigens ('N') [14, 26] and *GYP*Mur* alleles.

One GP.Hut (*GYP A-B-A*) donor who was Mi(a+) was not detected on this panel. The comparisons of Mi^a antigen results to *GYP*Mur* alleles revealed a variety of MNS hybrids.

For Kell, Duffy, Lutheran and Diego systems, serological assays are not routinely performed. Genotyping screens high-prevalence antigen negative donors, such as those who are k(−), Fy(a−), Lu(b−) and Di(b−). In addition, Di(a+) is an important antigen in the reagent red cell panels we provide for routine alloantibody screening. In this study, all the *DI*01* donors we tested in R_1R_1/ R_2R_2 group could join the reagent red cell donor programme. Dombrock is the only system without commercial antisera in Hemo Panel. Dombrock genotypes of reagent red cells provide information for identifying RBC alloantibodies in multiple alloimmunized patients [33].

AMEL and *ABO* results were concordant to the gender and ABO blood groups of tested donors. In ABO blood groups, the relatively common ABO subgroups are B_3 and A_{el} among Taiwan blood donors. Besides decisive SNPs of *A*, *B* and *O* alleles, we selected *ABO*AEL.04* and *ABO*B3.03* to increase this panel set. The limitation is unable to confirm other ABO subgroups, such as Axe and B_{el}. In Hemo Panel version II, the frequencies of the rs136337 are C allele: 0.58, A allele:0.42, and for rs2010235 are C allele: 0.02 and A allele: 0.98 of tested donors. Though these polymorphisms are insufficient as barcode genotypes, but are still useful to be aware of possible mix-ups when testing the samples with same red cell antigen profiles. And this is also an improvement compared with commercial genotyping kits after pilot study and assay of Hemo panel version I.

Several issues need to be taken into consideration when applying donor genotypes and predicted phenotypes: (a) there is complexity in the Kidd blood group system for both genotypes and phenotypes [34]. Null or weak alleles of *JK*01* or *JK*02* may have the same SNVs such as c.342-1G>A and c.130G>A. Detection of Jk(a+) in *JK*01W.01* allele may vary with typing reagents [29, 35], which increases the difficulty of predicting rare Kidd phenotypes using only genotypes. Confirmation of Kidd antigens by serology is important when issuing antigen-negative blood units. (b) It has been reported that GP.Mur is the prevalent type of glycophorin hybrids in Taiwan [21]. Hemo Panel can only detect *GYP*Mur*/*GYP*Bun* allele. Therefore, omissions of other glycophorin hybrids may occur. (c) Genuine discrepancy is caused by 'N' antigen of *MM* donors who also have *GYP*Mur* alleles [14, 26]. In this study, not all donors with *GYP*Mur* alleles showed 'N' antigen. It is possible that the expression of 'N' antigen is around the detection limits of commercial anti-N. The dilution of anti-N reagent is also suggested in clinical blood bank practice in Taiwan. The expression of 'N' antigen in different *GYP*Mur* donors needs further studies. (d) Over 99% of blood donors were RhD-positive. However, several reports have shown that variant alleles of *RHD* may be detected in weak D, DEL and D-negative phenotypes [32, 36, 37]. The detailed detection of D antigen should be introduced in routine D antigen testing including different epitope-specific anti-D antibodies and adsorption/elution method. (e) When providing antigen-negative blood units to recipients who have alloantibodies, it is suggested to avoid those with a genotype–phenotype discrepancy [12].

In conclusion, we developed a Hemo Panel for MassARRAY platform for genotyping the common blood group systems among blood donors in Taiwan. The concordance rates of common phenotypes and genotypes were over 99%. The assay is effective for the identification of reagent red cells for transfusion recipient investigations and for rare donor identification. Implementation of MassARRAY-based blood group genotyping and donor recruitment criteria for blood group genotyping are excellent tools with both clinical and research applications. Knowledge of genetic variations and associated blood groups in our donor population allows for issuing of proper blood units to patients to improve transfusion safety.

ACKNOWLEDGEMENTS

The authors appreciate Ms. Mo-Li Chen in Taipei blood centre, Taiwan Blood Services Foundation, for her help in the collection of the blood samples in pilot stage of the study. The authors are grateful to Mr. Matt, Shu-Ming Cheng and Mr. Chun-Yi Weng (Clinical lab of Feng Chi Biotech Crop) for providing great help in setting up the instruments, designing the useful Hemo Panel and analysing the preliminary genotyping data.

M.-H. Y. performed the research and wrote the first draft of the manuscript; M.-H. Y. and J.-W. C. designed the research study; J.-W. C., S.-T. W. and S.-M. H. supervised the research and Y.-J. C. reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

FUNDING INFORMATION

No funding.

ORCID

Meng-Hua Yang https://orcid.org/0000-0001-9280-0803

REFERENCES

1. Denomme GA, Anani WQ, Avent ND, Bein G, Briggs LB, Lapadat RC, et al. Red cell genotyping precision medicine: A conference summary. Ther Adv Hematol. 2017;8:277–91.
2. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med. 2015;372:793–5.
3. Compernolle V, Chou ST, Tanael S, Savage W, Howard J, Josephson CD, et al. Red blood cell specifications for patients with hemoglobinopathies: A systematic review and guideline. Transfusion. 2018;58:1555–66.
4. Klein HG, Flegel WA, Natanson C. Red cell transfusion: Precision versus imprecision medicine. JAMA. 2015;314:1557–8.
5. Flickinger C. REGGI and the American rare donor program. Transfus Med Hemother. 2014;41:342–5.
6. Tanaka E, Kusumi T, Takahashi H, Hirashima M, Tanaka M, Kimura K, et al. Defining and finding the rare donor. ISBT Sci Ser. 2014;9:168–70.
7. Revelli N, Villa MA, Paccapelo C, Manera MC, Rebulla P, Migliaccio AR, et al. The Lombardy rare donor programme. Blood Transfus. 2014;12:s249–55.
8. Flegel WA, Gottschall J, Denomme G. Implementing mass-scale red cell genotyping at a blood center. Transfusion. 2015;55:2610–5.
9. Guo Y, Busch MP, Seielstad M, Endres-Dighe S, Westhoff CM, Keating B, et al. Development and evaluation of a transfusion medicine genome wide genotyping array. Transfusion. 2019;59:101–11.

10. Bonet Bub C, Castilho L. ID CORE XT as a tool for molecular red blood cell typing. Expert Rev Mol Diagn. 2019;19:777–83.
11. Sasaki K, Suzuki Y, Isa K, Osabe T, Watanabe-Okochi N, Azuma F, et al. Genotyping of red cell blood groups by the liquid bead array system (LUMINEX). Japanese J Transfus Cell Ther. 2017;63:112–9.
12. Krog GR, Rieneck K, Clausen FB, et al. Blood group genotyping of blood donors: Validation of a highly accurate routine method. Transfusion. 2019;59:3264–74.
13. Meyer S, Vollmert C, Trost N, Brönnimann C, Gottschalk J, Buser A, et al. High-throughput Kell, Kidd, and Duffy matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry-based blood group genotyping of 4000 donors shows close to full concordance with serotyping and detects new alleles. Transfusion. 2014;54:3198–207.
14. Jongruamklang P, Gassner C, Meyer S, Kummasook A, Darlison M, Boonlum C, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of 36 blood group alleles among 396 Thai samples reveals region-specific variants. Transfusion. 2018;58:1752–62.
15. Orzinska A, Guz K, Brojer E. Potential of next-generation sequencing to match blood group antigens for transfusion. Int J Clin Transfus Med. 2019;7:11–22.
16. Jakobsen MA, Dellgren C, Sheppard C, Yazer M, Sprogøe U, et al. The use of next-generation sequencing for the determination of rare blood group genotypes. Transfus Med. 2019;29:162–8.
17. Schonewille H, Honohan Á, van der Watering LM, Hudig F, Te Boekhorst PA, Koopman-van Gemert AW, et al. Incidence of alloantibody formation after ABO-D or extended matched red blood cell transfusions: A randomized trial (MATCH study). Transfusion. 2016;56:311–20.
18. Lin-Chu M, Broadberry RE, Chang FJ. The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. Transfusion. 1988;28:350–2.
19. Chen JC, Lin TM, Chen YL, Wang YH, Jin YT, Yue CT, et al. RHD 1227A is an important genetic marker for RhDel individuals. Am J Clin Pathol. 2004;122:193–8.
20. Liu HM, Lin JS, Chen PS, Lyou JY, Chen YJ, Tzeng CH. Two novel *Jk*^{null} alleles derived from 222C>A in Exon 5 and 896G>A in Exon 9 of the *JK* gene. Transfusion. 2009;49:259–64.
21. Hsu K, Lin YC, Chang YC, Chan YS, Chao HP, Lee TY, et al. A direct blood polymerase chain reaction approach for the determination of GP.Mur (Mi.III) and other Hil+ Miltenberger glycophorin variants. Transfusion. 2013;53:962–71.
22. Metaxas-Bühler M, Metaxas MN, Sturgeon P. MNSs and Miltenberger frequencies in 211 Chinese. Vox Sang. 1975;29:394–9.
23. Boyle J, Thorpe SJ, Hawkins JR, Lockie C, Fox B, Matejtschuk P, et al. International reference reagents to standardise blood group genotyping: Evaluation of candidate preparations in an international collaborative study. Vox Sang. 2013;104:144–52.
24. Gassner C, Meyer S, Frey BM, Vollmert C. Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry-based blood group genotyping – The alternative approach. Transfus Med Rev. 2013;27:2–9.
25. Wei L, Lopez GH, Ji Y, Condon JA, Irwin DL, Luo GP, et al. Genotyping for Glycophorin *GYP(B-A-B)* hybrid genes using a single nucleotide polymorphism-based algorithm by matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry. Mol Biotechnol. 2016;58:665–71.
26. Wilson B, Grubor V, Lopez G, Hyland C, Liew Y, Flower R. Variant reactions with monoclonal anti-N in genotypically N-negative Gp. Mur (Mi III) positive Chinese & East Asian donors. ANZSBT Poster. 2010. https://anzsbt.org.au/wp-content/uploads/2018/06/ANZSBTposters2010_000.pdf.
27. Lin M, Yu L. Transfusion medicine. 4th ed. Wu-Nan Book; 2018.
28. Yang MH, Li L, Kuo YF, Hung YS, Yu LC, Hung CS, et al. Genetic and functional analyses describe a novel 730delG mutation in the *KEL* gene causing K_0 phenotype in a Taiwanese blood donor. Transfus Med. 2011;21:318–24.
29. Wester ES, Storry JR, Olsson ML. Characterization of Jk(a+^{weak}): A new blood group phenotype associated with an altered *JK*01* allele. Transfusion. 2011;51:380–92.
30. Guelsin GA, Horn T, Crowley JE. Jk nt130G>A found on both *JK*01* and *JK*02* alleles in US and Brazilian populations. Transfusion. 2013;53:163A.
31. Flegel WA, Gottschall JL, Denomme GA. Integration of red cell genotyping into the blood supply chain: A population-based study. Lancet Haematol. 2015;2:e282–8.
32. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: A review and guidance for interpreting the RhD blood type using the *RHD* genotype. Br J Haematol. 2017;179:10–9.
33. Scharberg E, Rink G, Portegys J, Rothenberger S, Gillhuber N, Richter E, et al. The impact of using genotyped reagent red blood cells in antibody identification. Transfus Med Hemother. 2018;45:218–24.
34. Hamilton JR. Kidd blood group system: Outwardly simple with hidden complexity. ISBT Sci Ser. 2019;14:3–8.
35. Wu PC, Chyan TW, Feng SH, Chen MH, Pai SC. Genotyping and serotyping profiles showed weak Jk^a presentation for previously typed as Jk$_{null}$ donors. Vox Sang. 2019;114:268–74.
36. Okuda H, Kawano M, Iwamoto S, Tanaka M, Seno T, Okubo Y, et al. The *RHD* gene is highly detectable in RhD-negative Japanese donors. J Clin Invest. 1997;100:373–9.
37. Perez-Alvarez I, Hayes C, Hailemariam T, Shin E, Hutchinson T, Klapper E. *RHD* genotyping of serologic RhD-negative blood donors in a hospital-based blood donor center. Transfusion. 2019;59:2422–8.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Yang M-H, Chen J-W, Wei S-T, Hou S-M, Chen Y-J. The efficacy of ethnic specific blood groups genotyping for routine donor investigation and rare donor identification in Taiwan. Vox Sang. 2021;1–10.

Received: 30 March 2021 | Revised: 16 May 2021 | Accepted: 16 May 2021

DOI: 10.1111/vox.13160

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Challenges in antibody titration for ABO-incompatible renal transplantation

Htar Kahlyar[1] | David Roxby[2] | Tony Badrick[1] | Thiru Vanniasinkam[3]

[1]Royal College of Pathologists of Australasia, Sydney, New South Wales, Australia

[2]College of Medicine and Public Health, Flinders University, South Australia, Australia

[3]School of Biomedical Sciences, Charles Sturt University, New South Wales, Australia

Correspondence
Thiru Vanniasinkam, School of Biomedical Sciences, Charles Sturt University, NSW 2650, Australia.
Email: tvanniasinkam@csu.edu.au

Abstract

Background and Objectives: Accurate and regular monitoring of anti-A and anti-B titres pre- and post-transplantation plays a crucial role in the clinical management of patients receiving ABO-incompatible renal transplants. There is no standardized protocol or an external quality assurance program (EQA) currently available for this testing in Australia. The aim of this study was to investigate the diversity of techniques, test platforms and reagents that were currently in use in various laboratories with the aim of developing an EQA.

Materials and Methods: An online survey was sent to the participants enrolled with the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) to assess their interest in participation in the pilot study. A total of 24 participants who expressed interest were sent the group O plasma, A_1, A_2 and B cells to perform ABO titration using their own methods.

Results: Participants reported a wide range of titre results, from 8 to 1024 for the anti-A titre using A_1 cells, from 2 to 128 for anti-A titre using A_2 cells and from neat to 32 for anti-B titre using B cells.

Conclusion: There was a wide variation in titre results between and within different technologies. These findings demonstrate the need for an ABO titration EQA. Development of a standard technique and participation in an EQA program should, over time, reduce variation and enable transferrable results across testing centres, which will assist in consistent clinical interpretation and better outcomes for patients.

KEYWORDS
ABO, external quality assurance, renal transplantation, titration

INTRODUCTION

Kidney transplantation is the best possible treatment to improve the quality of life of patients with end-stage kidney disease [1]. The 43rd annual ANZDATA report, published in 2020, stated that there were 3229 new patients requiring renal replacement therapy in Australia with an overall incidence rate of 127 per million population [2]. According to Kidney Health Australia, by the end of September 2018, 1003 patients were on the waiting list for a kidney transplant with the median waiting period of 3 years [3]. However, longer waiting times of up to 7 years had also been reported, and in 2016, only 42% of the patients on the waiting list were transplanted, and eight patients died while waiting for a kidney transplant [3]. Transplant Australia reported that kidney is the most in demand organ due to the fact that the patients can survive on dialysis while waiting for the donor kidney [4].

Due to the high number of patients requiring kidney transplantation and issues with obtaining kidneys from ABO-compatible donors, ABO-incompatible transplantations (ABOi) are often performed. However, accurate and regular monitoring of anti-A and anti-B titres pre- and post-transplantation plays a crucial role in the clinical management

of these patients in order to avoid an antibody-mediated rejection (AMR). AMR leads to endothelial damage, intravascular thrombosis and compromised oxygen supply to the transplanted kidney [5]. The danger of graft loss due to AMR is a significant concern in ABOi renal transplantation [6]. In addition, ABOi transplant is linked to increased rates of patient morbidity and mortality due to infectious complications, caused by desensitization therapy before transplantation [7]. However, desensitization increases the donor pool [8], and compared to patients on the waiting lists or dialysis, patients who received ABOi renal transplant have better clinical outcomes [7].

Strategies to modulate immune responses in ABOi transplantation include, desensitization therapies, immunoadsorption [9, 10] and therapeutic plasma exchange (TPE) to remove circulating antibodies [11]. Some of these approaches have significant disadvantages, for example, TPE not only removes antibodies but also complements and coagulation factors such as fibrinogen and FXIII [12]. Rituximab is an anti-CD20 monoclonal antibody used to suppress B cells as part of the immunosuppressive strategy before ABOi kidney transplantation [13]. In addition, immunosuppressive medications such as IVIg are utilized to prevent rejection [14]. Importantly, post transplantation, ABO titres are monitored closely to prevent antibody-mediated rejection and to commence treatment in cases of rising titre levels [15].

There are reports in the literature of successful transplantation in the presence of high antibody titres [16] and reports of accommodation in some ABOi renal transplants [17]. An accommodation phenomenon occurs when the graft organ was not rejected in spite of the presence of recipient antibodies against the antigens on the donated kidney [18]. However, antibody titration is still a vital test, the results of which can be used to implement treatments to decrease anti-A and anti-B titres before transplantation and also to maintain low titre levels post transplantation [19]. In addition, measurement of ABO titres is important to predict engraftment in ABO incompatible stem cell transplantation, to monitor titre levels in transfusion reaction investigations, haemolytic disease of the newborn and to detect the ABO subgroups [7].

Antibody titration is difficult to standardize due to variations in protocols, testing platforms and endpoint cut off among different laboratories [20]. Currently, there is no external quality assurance (EQA) program available for ABO titration in Australia, and there is no published evidence of internationally recognized standard protocols or guidelines.

No similar Australasia-wide studies have been conducted previously to assess the level of inter-laboratory variability in the determination of ABO antibody titration. The aims of this study were to investigate (1) the various techniques and reagents used by the medical laboratories for ABO antibody titration in Australasia and (2) the requirements for the development and introduction of an EQA program. This study was approved by Charles Sturt University Human Research Ethics Committee, approval number H19026.

MATERIALS AND METHODS

Fresh frozen plasma packs and red cell units were received from the Australian Red Cross Lifeblood (ARCL). All donations were tested by Lifeblood and found to be serologically negative for HIV, HCV and HBsAg. The red cell units were phenotyped by the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) for their A_1 status with anti-A_1 lectin, *Dolichos biflorus*. The plasma sample was prepared by thawing one unit of donor group O fresh frozen plasma at 56°C, mixing it and transferring into 4-mL EDTA tubes. The red cell samples were prepared by resuspending A_1, A_2 and B donor red cells at a 0.2% concentration in a red cell preservative solution Celpresol™ (Immulab, Victoria, Australia) and aliquotted into 2-mL EDTA tubes.

Participants

In November 2018, an online survey (Appendix S1) was sent out by the RCPAQAP Transfusion group to over 400 laboratories that were enrolled in the RCPAQAP General Transfusion program, to gauge their interest in participation in an ABO antibody titration pilot study. Due to the location of the RCPAQAP in Australia, most of the laboratories enrolled in the transfusion programs were from Australasia. A total of 24 laboratories from six countries (Australia (16), New Zealand (2), Malaysia (3), Singapore (1), Oman (1) and United Kingdom (1)) returned results for the pilot study. The number of participating laboratories was small because renal transplantation is a highly specialized area.

Samples provided

In April 2019, RCPAQAP Transfusion sent group O plasma, A_1, A_2 and B red cells with a second questionnaire (Appendix S2) to those laboratories. The second questionnaire included a list of 13 questions, seeking information about the testing platforms used (tube or column agglutination technology), manual or automated technique, direct, indirect or both methods, diluent used, incubation temperatures (room temperature, 4°C or 37°C), use of dithiothreitol (DTT)-treated plasma and endpoint used for titration.

The laboratories were provided with a 4 mL group O plasma sample and 2 mL samples of A_1, A_2 and B cells. They were asked to perform an antibody screen on the plasma and ABO titration against all three red cells samples using their own routine methods and to report all results from all their methods if they routinely performed more than once, within 2 weeks.

Analysis of data

Based on the aims of this project and the small number of laboratories that participated in the study, it was not appropriate to undertake statistical analysis of the data. The data obtained were tabulated in excel for analysis and graphs prepared.

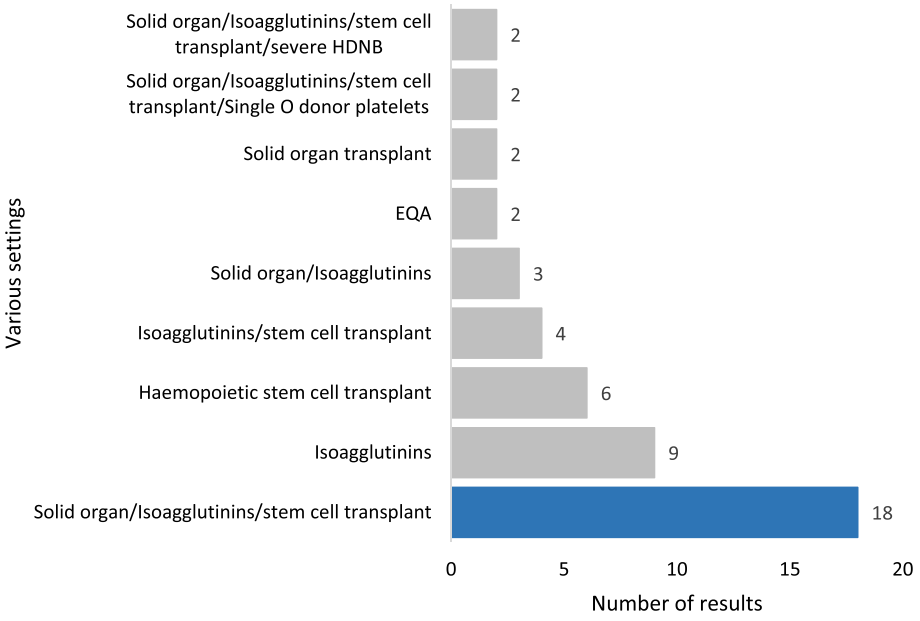


FIGURE 1 ABO titration results obtained under various settings from 24 participants. Fourteen of these laboratories performed ABO titration, using more than one method in multiple settings. The highest number of participants performed ABO titration in solid organ, isoagglutinin detection and stem cell transplant settings [Colour figure can be viewed at wileyonlinelibrary.com]

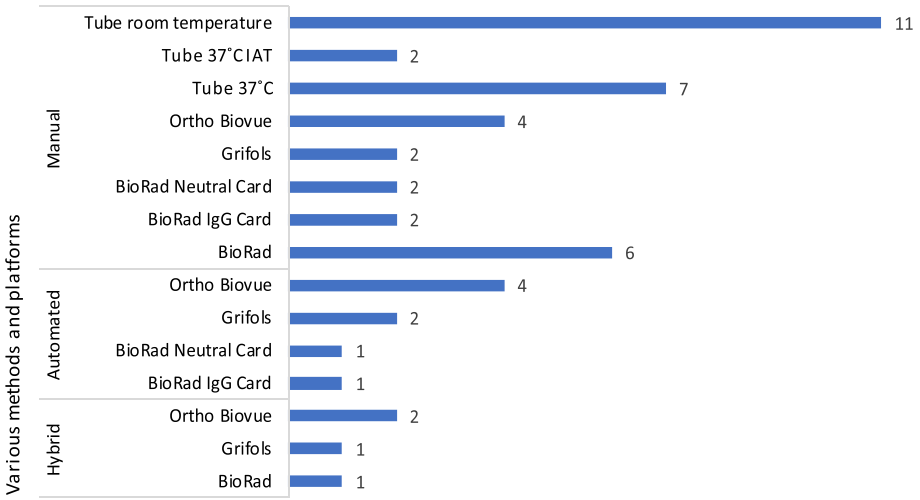


FIGURE 2 Test methods used to perform ABO titration. Tube was the most commonly used method, and participants performed ABO titration using tube method at both room temperature and at 37°C [Colour figure can be viewed at wileyonlinelibrary.com]

RESULTS

Test data

Twenty-four laboratories returned results, which is 86% (24/28) of those that initially expressed their interest. Of the 24 laboratories, 14 (58%) laboratories reported multiple results as they performed ABO titrations using multiple methods and in different settings such as solid organ transplant, isoagglutinin detection, stem cell transplant and detection of low titre single O donor platelets. Therefore, the total number of results reported was 48. A total of 22 out of 24 (92%) participants submitted antibody screen results on the plasma samples and reported the screen as negative.

Laboratory settings for performing ABO titration

As shown in Figure 1, participants reported that ABO titration was performed in various laboratory settings such as ABO-mismatched solid organ transplant, haemopoietic stem cell transplant, determination of isoagglutinin levels as a screen of immunological competence and severe haemolytic disease of the newborn.

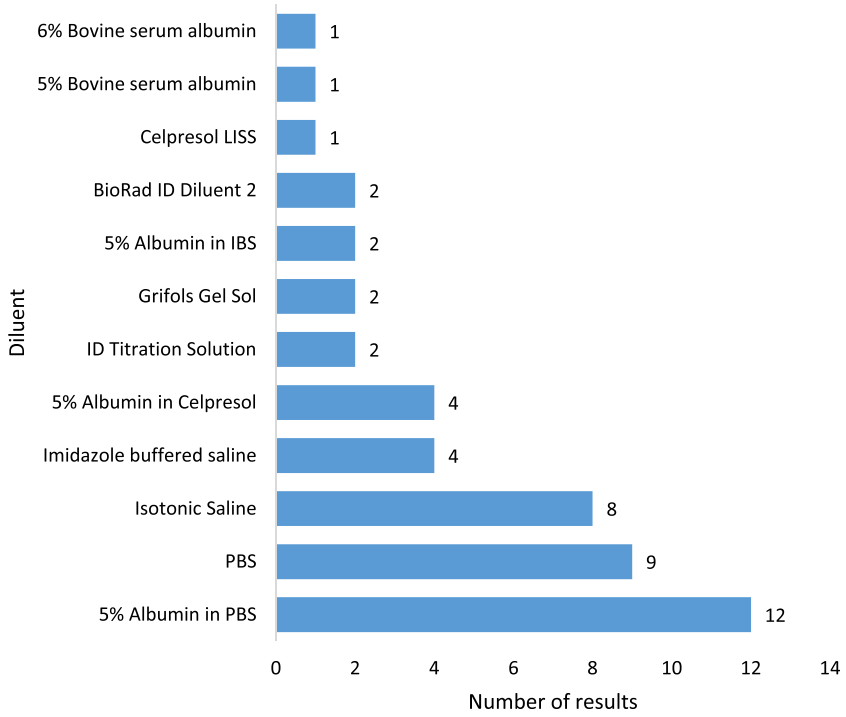


FIGURE 3 Various diluents used to perform ABO titration. The figure reveals that 12 different diluents were used, some with potentiators such as albumin and some without. The most commonly used diluent was 5% albumin in PBS (phosphate-buffered saline) [Colour figure can be viewed at wileyonlinelibrary.com]

Techniques used for ABO titration

A wide range of methods and platforms were used by participants to perform ABO titration. As shown in Figure 2, the most widely used platforms reported by participants were tube and column agglutination technology cards such as CAT-gel, (Bio-Rad, Gladesville, New South Wales, Australia and Grifols, Clayton South, Victoria, Australia) or CAT-glass beads, (Biovue, Ortho Clinical Diagnostics Mulgrave, Victoria, Australia). Participants also reported whether they used manual method, automated analyser or a hybrid method where manual serial dilutions were prepared, and the cards were read using an automated analyser. A total of 20/48 (42%) responses reported using tube method, 6/48 (13%) responses reported the manual Bio-Rad method and 4/48 (8.3%) responses reported a manual Ortho Biovue method.

Diluents used

Twelve different diluents were used for preparation of serial dilutions. As shown in Figure 3, 12/48 (25%) responses reported using 5% albumin in phosphate-buffered saline (PBS), 9/48 (19%) reported using PBS and 8/48 (17%) responses reported using isotonic saline.

Incubation temperature and DTT treatment

When participants were asked to report the incubation temperature of the ABO titration test, 31/48 (65%) responses reported incubation at 37°C and 17/48 (35%) using a room temperature incubation. None of the participants reported performing DTT treatment of plasma to differentiate between IgG and IgM.

Endpoint used to report titres

From the responses received, it was clear that a wide range of endpoints were used by participants to report the ABO titre, as shown in Figure 4. The most common endpoints used were 'last dilution giving 1+ reaction' (19/48) or 'last dilution giving 2+ reaction' (11/48). A total of 9/48 (19%) responses reported using a different endpoint for renal transplants compared to other clinical settings, 5/48 (10%) responses reported using an endpoint of 'last dilution giving 2 + reaction' to report renal transplant setting and 'last dilution giving 1+ reaction' for all other clinical settings and 4/48 (8%) used the 'last dilution giving 1+ reaction' to report renal transplant and 'last dilution giving 0.5 reaction' to report haematopoietic stem cell transplants.

Anti-A_1 titre results using A_1 cells in comparison with method platforms

As shown in Figure 5, 48 responses were received for titre results against A_1 cells, ranging from 8 to 1024 using different test methods such as Bio-Rad, Ortho Biovue, Grifols and tube.

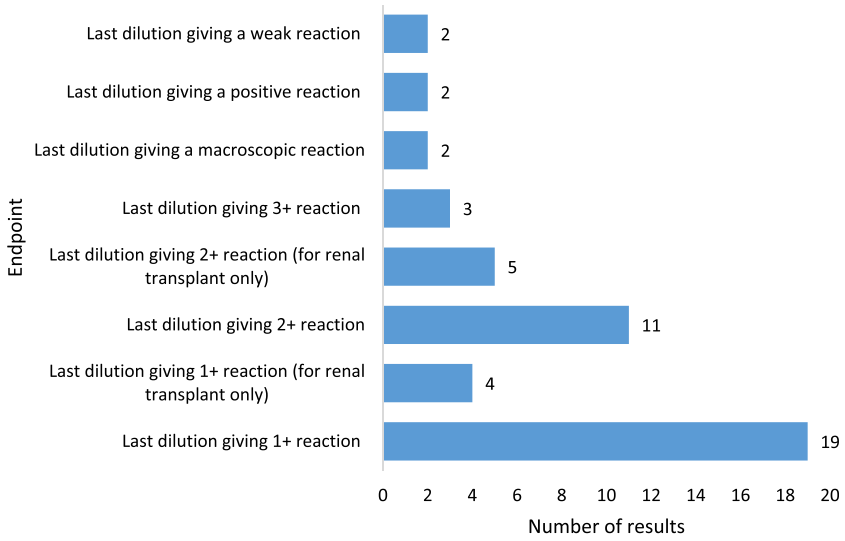


FIGURE 4 The endpoints used to report ABO titres. The figure reveals that the most commonly used endpoint is last dilution giving 1+ reaction, and some participants used a different endpoint to report ABO titres for renal transplant [Colour figure can be viewed at wileyonlinelibrary.com]

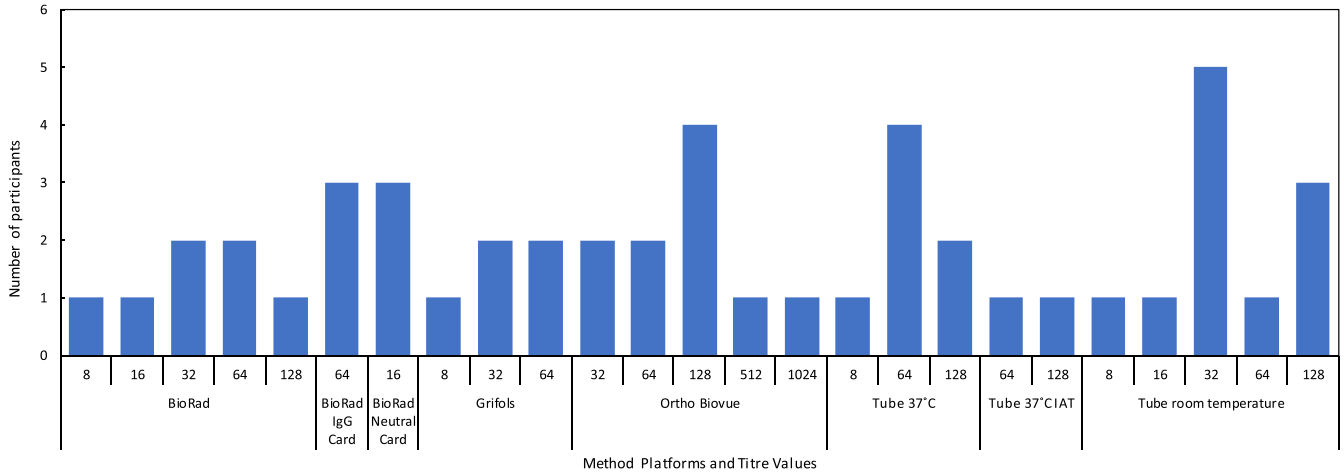


FIGURE 5 Anti-A_1 titre results using A_1 cells. Comparison of various protocols/platforms such as BioRad, Grifols, Biovue and tube. The figure demonstrates variation in titre results within and between methods [Colour figure can be viewed at wileyonlinelibrary.com]

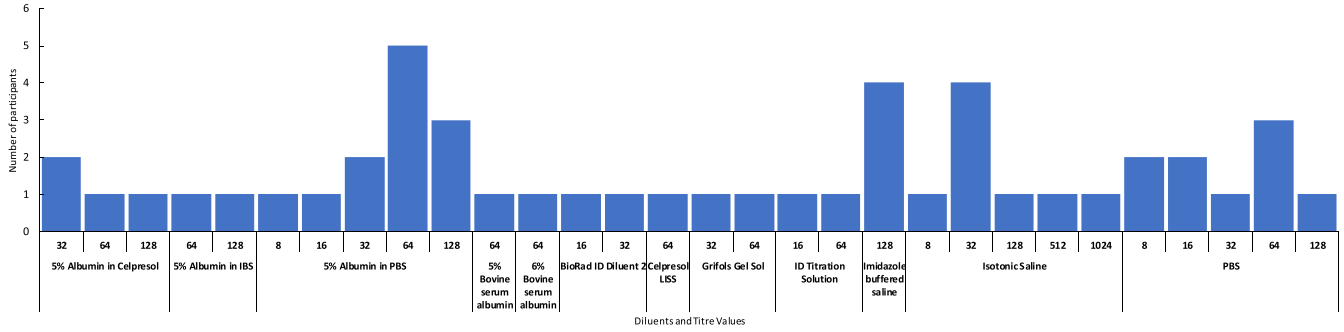


FIGURE 6 Anti-A_1 titre using A_1 cells. Comparison of various diluents. There is a difference in titre results even when the same diluent is used by different laboratories [Colour figure can be viewed at wileyonlinelibrary.com]

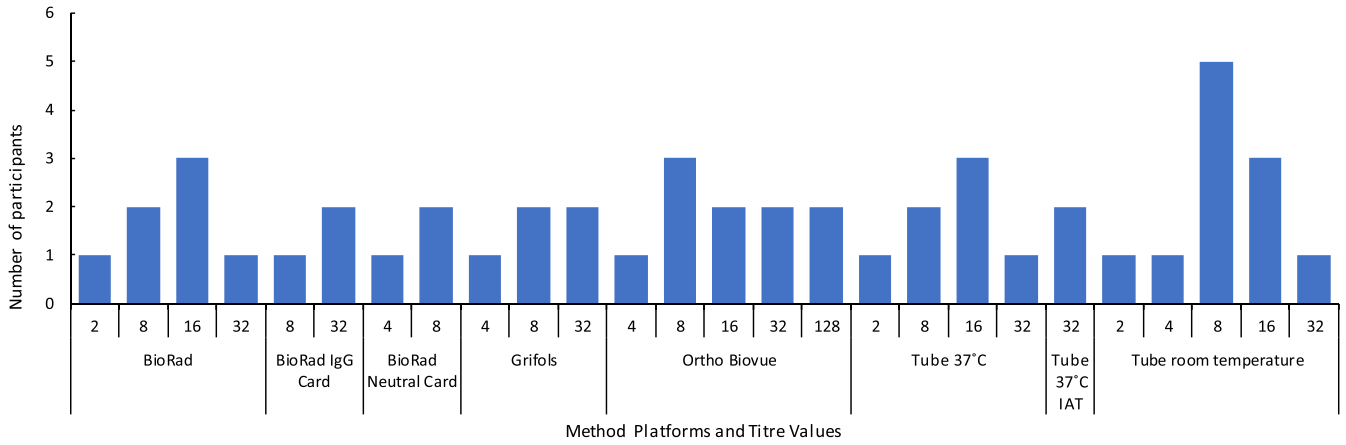


FIGURE 7 Anti-A titre using A_2 cells in comparison with method platforms such as BioRad, Grifols, Biovue and tube. The figure shows varying titre results within and between methods [Colour figure can be viewed at wileyonlinelibrary.com]

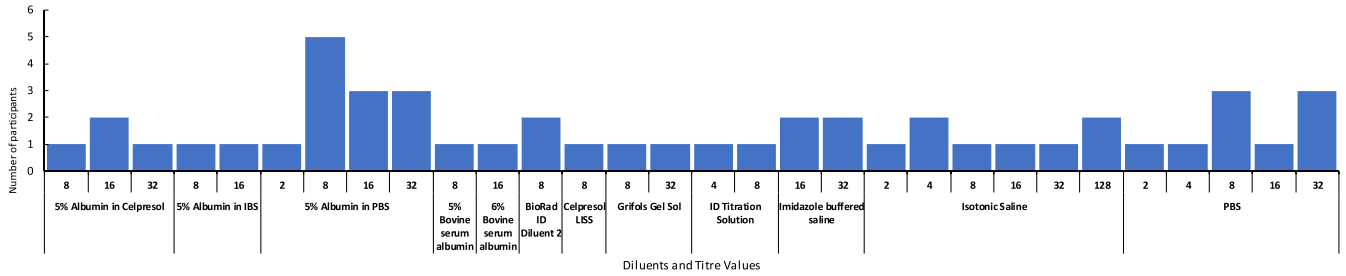


FIGURE 8 Anti-A titre using A_2 cells in comparison with various diluents. There is a difference in titre results even when the same diluent is used by different laboratories [Colour figure can be viewed at wileyonlinelibrary.com]

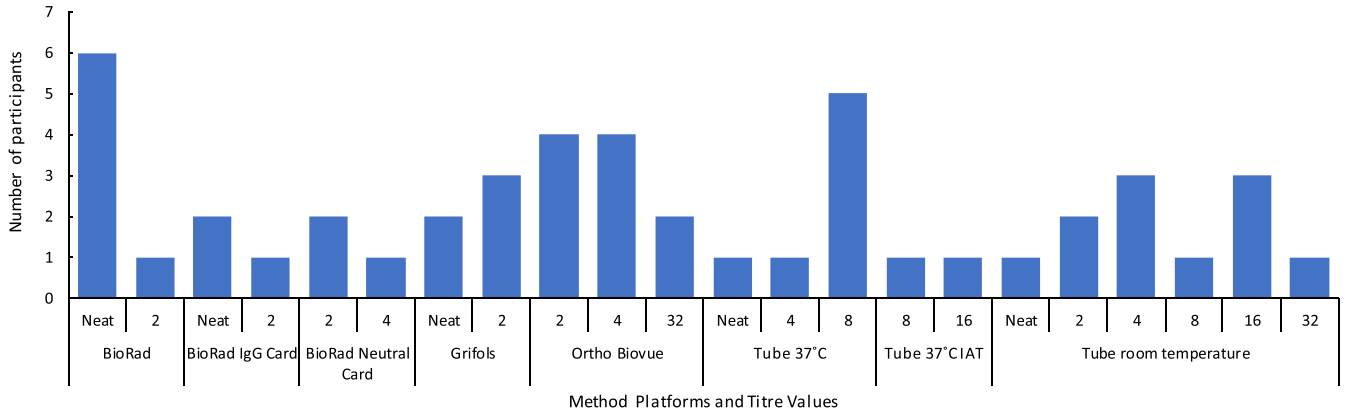


FIGURE 9 Anti-B titre in comparison with method platforms such as BioRad, Grifols, Biovue and tube. The figure reveals varying titre results within and between methods [Colour figure can be viewed at wileyonlinelibrary.com]

Anti-A_1 titre results using A_1 cells in comparison with diluents

Forty-eight responses were received for anti-A_1 titre results with A_1 cells ranging from 8 to 1024 using a wide range of diluents. As shown in Figure 6, the most commonly used diluents were 5% albumin in PBS, isotonic saline and imidazole-buffered saline.

Anti-A titre results using A_2 cells in comparison with method platforms

For anti-A titre with A_2 cells, as shown in Figure 7, a total of 48 responses were received, ranging from 2 to 128 using either Bio-Rad, Ortho Biovue, Grifols or tube.

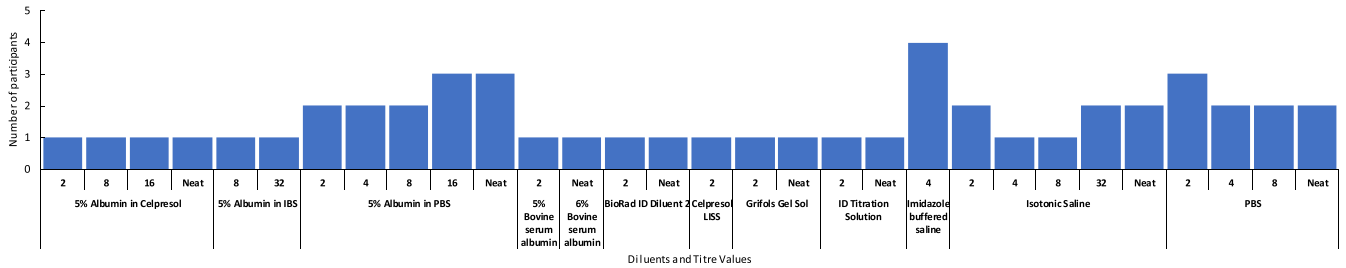


FIGURE 10 Anti-B titre in comparison with various diluents. There is a difference in titre results even when the same diluent is used by different laboratories [Colour figure can be viewed at wileyonlinelibrary.com]

Anti-A titre results using A_2 cells in comparison with diluents

A total of 48 responses were received for anti-A titre results with A_2 cells ranging from 2 to 128 using a wide range of diluents. As shown in Figure 8, the most commonly used diluents are 5% albumin in PBS and PBS.

Anti-B titre results in comparison with method platforms

As shown in Figure 9, 48 responses were received for anti-B titre results ranging from neat to 32 using either Bio-Rad, Ortho Biovue, Grifols or tube.

Anti-B titre results in comparison with diluents

A total of 48 responses were received for anti-B titre results using a wide range of diluents. As shown in Figure 10, the most commonly used diluents are imidazole-buffered saline, 5% albumin in PBS and PBS.

DISCUSSION

There are multiple factors that can affect the determination of antibody titres including testing platform, diluents, incubation time, strength of reaction cut off, testing phase, skills and experience of the personnel [21]. The results of the first survey questionnaire indicated wide inter-laboratory variation in techniques used (tube or column technology), process (manual or automated), incubation temperatures (room temperature or 37°C), methods (direct or indirect), red cell diluents and titration endpoints used. Critical findings from the second questionnaire and titration pilot highlighted not only the diversity of techniques used but also the wide titre ranges reported by the participants.

The overall titre results against A_1, A_2 and B red cells varied widely depending on the method platform and diluents used ranging from 8 to 1024 for anti-A_1, 2 to 128 for anti-A and 0 to 32 for anti-B titre. Nine responses (19%) indicated that a different titre endpoint was used for renal transplant while using a different endpoint for all other settings. Upon further follow-up with the participants who contributed these responses, they reported that it was based on local protocols and directives from their clinicians rather than on the clinical guidelines.

In their study, Nayak et al., performed titration on 48 group O blood samples using five different methods such as immediate spin tube, antihuman globulin phase (AHG) tube, gel column card without DTT treatment, gel column card with DTT treatment and the solid phase technique. They reported that anti-A and anti-B titres are the highest in the tube method by AHG phase and DTT treatment reduced both anti-A and anti-B titre values in almost 50% of the samples [22]. They also found poor consensus between AHG phase tube method and gel column agglutination for both anti-A and anti-B titres [22].

In comparison, participants in this study, who used BioVue glass bead column agglutination technology reported higher results for anti-A_1, anti-A and anti-B titres. In addition, none of the participants performed DTT treatment of plasma. DTT is used to cleave disulfide bonds of IgM antibodies in order to measure the strength and concentration of remaining IgG antibodies in patient's plasma [23]. The British Transplantation Society's ABOi kidney transplantation guidelines recommend differentiation of IgM and IgG immunoglobulin classes for ABO antibodies [24]. Most UK transplant centres titrate both IgG and IgM to guide reduction of ABO antibodies but use the higher result (Personal communication, UK NEQAS). In Australia, laboratories are mostly focussed on the measurement of IgG antibodies as they react at 37°C and are considered more clinically significant, whereas IgM antibodies react preferentially below 37°C [25]. On the other hand, Kim et al., have shown that patients had the same clinical outcome regardless of high or low IgG titres if pre-transplant IgM titres were lowered to 4 or less [26].

Other researchers have also reported noticeable differences in titre results based on detection method used. The manual tube method was the most widely used; however, this is time consuming and produces variable results within and between laboratories [27]. The research team at Ajou University School of Medicine performed antibody titration on 20 samples using tube room temperature, tube IAT, CAT gel and a flow cytometry method (FCM) [28]. They reported

that for all blood groups, FCM assay with anti-IgM produced the highest titre results; for A and B blood groups, tube IAT showed the highest titres, and for anti-A titre in blood group O, CAT gel had the highest results.

Due to the labour intensiveness of the tube method and subjective interpretation of results, researchers investigated the fully automated titration method and suggested that it is a reliable technique and may reduce the variation encountered in the conventional tube method [23]. Lally et al., showed good correlation between comparison of manual gel column technology with the automated solid phase analyser [29]. However, further studies on the clinical significance of titres are required before a fully automated system can reliably replace manual techniques [30]. The researchers who compared the erythrocyte magnetized technology (EMT) with tube and Bio Vue column methods reported higher results with EMT, which were especially inconsistent for IgG [30].

In our study, the large degree of variation in titres reported and variability in technique, diluent used, incubation temperature, titre endpoints and results reported by the participants are of concern as ABO titre results are a critical part of the decision-making process in transplant eligibility, treatment and monitoring the efficacy of therapeutic intervention [31]. The levels of pre-transplant anti-A_1 and anti-B titres influence the planning of immunosuppression therapy and also assist in establishing the most suitable time for transplantation [5]. Accurate measurement of ABO titres is not only crucial to limit unnecessary deferral of patients who otherwise would be eligible for transplant but also to save time and resources on unwarranted pre-transplant interventions to lower titre levels [22].

Based upon the finding of this study, it is clear that ABO titre results are not transferrable between different laboratories, and it is also difficult to compare the cost-effectiveness and clinical outcomes across transplant centres. It is possible for a kidney transplant patient to be admitted at different hospitals with a high variation in titre result which can lead to treatment with unnecessary high-risk procedures as well as unnecessary deferral of the transplant procedure [21]. In Australia, clinicians ensure that the ABO titration test is repeated in their laboratory when the patients are referred from different hospitals, to enable the same titre cut off point base to be used to make treatment decisions on the treatment plans. However, the equity of transplant eligibility across different transplant institutions is a concern for the clinicians due to the high variability of titre results [22]. The Transplantation Society of Australia and New Zealand (TSANZ) defines equity as an equal opportunity for every patient who requires kidney transplant to receive one [32]. In addition, patients should be able to have the same eligibility to transplant regardless of the transplant centre they were admitted to in Australia and the methods used to perform ABO titration. Therefore, it is critical to implement a standardized method in order to achieve reproducible results that can be compared across different clinical centres and to assist in consistent clinical interpretation [21].

Researchers in New Zealand attempted to investigate the possibility of introducing a standardized method by developing the kodecyte assay, but clinical relevance and further trials are required before it can fully replace contemporary ABO titration methods [33]. Other EQAs such as the United Kingdom National External Quality Assurance Scheme (UK NEQAS) investigated ABO titration methods in 2016 and reported a tremendous amount of variation between and within technologies used. A standard protocol based on BioRad technology was recommended; however, a standardized ABO titration protocol has not yet been fully achieved as for the clinicians; there are risks in changing practice as the evidence that cut-off values are effective is based on testing using a particular method and the transition to another standard method requires various considerations (Personal communications, UK NEQAS).

Similar to the UK NEQAS experience, RCPAQAP has encountered some challenges in introducing an EQA for ABO titration due to the significant amount of variation used by participants in method, testing phase, strength of endpoint, diluents and red cells. The next step would be to send out another ABO titration pilot with standard protocol to investigate whether standardizing certain aspects of the titration method reduces the high variation in the results that currently exist. It is also essential to engage the renal transplant society, to cooperate with various groups including renal transplant surgeons, physicians, immunologists and laboratory scientists to create an Advisory Committee for the ABO Titration EQA program. In future, it will be prudent to collaborate with the International Society of Blood Transfusion (ISBT) and other EQA providers to highlight to the clinicians the variations in titration results within and between methods and to promote international standardization of ABO titration procedures. In addition, it would be useful to obtain further information on the types of solid organ transplants being undertaken and specific methods used for ABO titration in each of these setting. Further studies should also look at increasing the number of participating laboratories by collaborating with other EQA providers.

This study was undertaken to review the current status of ABO titration protocol used by the laboratories in the field of ABOi renal transplant as this has not been undertaken previously in Australia. The findings highlight the need for standardization and establishment of an EQA and make an important contribution to the field of ABO titration in ABOi renal transplantation. Future studies involving more laboratories in other countries could also be helpful in obtaining more data to examine ABOi renal transplantations further. This would also enable collaboration with the various clinical groups to potentially pave the way toward global standardization.

There is a substantial degree of diversity that exists in ABO titration procedures, in diagnostic laboratories including techniques, diluents, red cells and strength of reaction cut off. Our study has provided information around the current status of ABO titration procedures in Australasia. Implementation of an EQA for ABO titration would allow for the objective peer assessment of the methods used currently. A goal for the community would be the standardization of methods so that there was a consistent quality of all reported results in this critical area of medicine.

ACKNOWLEDGEMENTS

We would like to acknowledge staff in all the laboratories who participated in this research. We would like to thank Jenny White (UK NEQAS) for her help with this study.

H.K designed the study, investigated and analysed the data and contributed in writing the final version of the paper. D.R., T.B. and T.V. designed the study and contributed in writing the final version of the paper.

CONFLICT OF INTEREST

None declared.

ORCID

Thiru Vanniasinkam https://orcid.org/0000-0001-9318-3982

REFERENCES

1. Jha PK, Bansal SB, Sethi SK, Jain M, Sharma R, Nandwani A, et al. ABO-incompatible renal transplantation in developing world—crossing the immunological (and mental) barrier. Indian J Nephrol. 2016;26:113–8.
2. ANZDATA. ANZDATA 43rd Annual Report 2020. Incidence of RRT in ESKD. anzdata.org.au. Australia and New Zealand Dialysis & Transplant Registry. 29th September 2020. https://www.anzdata.org.au/report/anzdata-43rd-annual-report-2020-data-to-2019/. Accessed 11 Jan 2021.
3. Kidney Health Australia. Kidney fast facts factsheet. Kidney.org.au. 2020. https://kidney.org.au/resources/factsheets-and-photosheets/kidney-fast-facts-factsheet. Accessed 20 Nov 2018.
4. Transplant Australia. Frequently asked questions. transplant.org.au. 2021. https://transplant.org.au/faq/. Accessed 9 Jan 2021.
5. Assing K, Sprogoe U, Nielsen C, Rasmussen M, Yazer M, Bistrup C. Increased but stable isoagglutinin titers in hemodialysis patients. J Nephrol. 2019;32:121–7.
6. Ali T, Broering D, Aleid H, Brockmann J, Alhumaidan H, Hammad E, et al. ABO incompatible kidney transplantation: the Saudi experience. Saudi J Kidney Dis Transpl. 2019;30:655–62.
7. Ko EJ, Yu JH, Yang CW, Chung BH, the Korean Organ Transplantation Registry Study Group. Clinical outcomes of ABO- and HLA-incompatible kidney transplantation: a nationwide cohort study. Transplant Int. 2017;30:1215–25.
8. Pandey P, Setya D, Sinha V, Bhatt A, Devra A, Chaudhary A, et al. Therapeutic apheresis in ABO-incompatible kidney and liver transplantation: a single-center experience of 50 patients. Ther Apher Dial. 2021;25:103–17.
9. Agrawal S, Chowdhry M, Makroo RN, et al. Therapeutic immunoadsorption and conventional plasma exchange in ABO-incompatible renal transplant: an exculpatory evidence. Cureus. 2019;11:e4787.
10. Baek CH, Kim H, Yang WS, et al. Clinical significance of isoagglutinin titre with the current desensitization protocol in ABO-incompatible kidney transplantation. Nephrol Ther. 2019;24:654–60.
11. Naganuma T, Takemoto Y, Hanaoka A, Uchida J, Nakatani T. Selective plasma exchange in abo-incompatible kidney transplantation: efficacy of partial substitution with fresh frozen plasma. Nephrol Dial Transplant. 2019;34. https://doi.org/10.1093/ndt/gfz103.SP787.
12. Hanaoka A, Naganuma T, Takemoto Y, et al. Difference in rate of decline of isoagglutinin titers between selective plasma exchange with and wuthout fresh frozen plasma before abo-incompatible kidney transplantation. Kidney Int. 2019;4:S39-S.
13. Maenosono R, Unagami K, Kakuta Y, Furusawa M, Okumi M, Azuma H, et al. Association between response to rituximab and antibody-mediated rejection in ABO-incompatible living kidney transplantation. Int J Urol. 2019;26:1114–20.
14. Shah Y, Almeshari K, Broering D, Aleid H, Brockmann J, Alhumaidan H, et al. ABO-incompatible kidney transplantation: low rates of infectious complications and excellent patient survival. Transplant Proc. 2019;51:512–6.
15. Shah BV, Rajput P, Virani ZA, Warghade S. Baseline anti-blood group antibody titers and their response to desensitization and kidney transplantation. Indian J Nephrol. 2017;27:195–8.
16. Ray DS, Thukral S. ABO-incompatible renal transplantation with high antibody titer: a case report. Am J Case Rep. 2017;18:1073–6.
17. Garcia de Mattos Barbosa M, Cascalho M, Platt JL. Accommodation in ABO-incompatible organ transplants. Xenotransplantation. 2018;25:e12418.
18. Hourmant M, Figueres L, Gicquel A, Kimmel C, Garandeau C. New rules of ABO-compatibility in kidney transplantation. Transfus Clin Biol. 2019;26:180–3.
19. Bhangale A, Pathak A, Pawar S, Jeloka T. Comparison of antibody titers using conventional tube technique versus column agglutination technique in ABO blood group incompatible renal transplant. Asian J Transfus Sci. 2017;11:131–4.
20. Khalili I, Koch M, Thaiss F, Plaetke R, Bruegger J, Peine S. Systematic comparison of IgM and IgG ABO antibody titers by using tube and gel card techniques and its relevance for ABO-incompatible kidney transplantation. Clin Lab. 2017;63:1393–401.
21. Bachegowda LS, Cheng YH, Long T, Shaz BH. Impact of uniform methods on Interlaboratory antibody titration variability: antibody titration and uniform methods. Arch Pathol Lab Med. 2017;141:131–8.
22. Nayak S, Makroo RN, Prakash B, Chandra T, Agrawal S, Chowdhry M, et al. Comparative evaluation of five different methods of anti-ABO antibody titration: an aid for ABO-incompatible organ transplants. Ther Apher Dial. 2019;23:86–91.
23. Matsuura H, Akatsuka Y, Matsuno T, Sugiura Y, Arakawa S, Oikawa S, et al. Comparison of the tube test and column agglutination techniques for anti-A/-B antibody titration in healthy individuals. Vox Sang. 2018;113:787–94.
24. British Transplantation Society. Guidelines for living donor kidney transplantation. British Transplantation Society. 2021. https://bts.org.uk/wp-content/uploads/2018/07/FINAL_LDKT-guidelines_June-2018.pdf. Accessed 8 Dec 2020.
25. Flegel WA. Pathogenesis and mechanisms of antibody-mediated hemolysis. Transfusion. 2015;55(Suppl 2):S47–58.
26. Kim H, Choe W, Shin S, Kim YH, Han DJ, Park SK, et al. ABO-incompatible kidney transplantation can be successfully conducted by monitoring IgM isoagglutinin titers during desensitization. Transfusion. 2020;60:598–606.
27. Adkins BD, Arnold Egloff SA, Fahey-Ahrndt K, Kjell AL, Cohn CS, Young PP. An exploration of the advantages of automated titration testing: low inter-instrument variability and equivalent accuracy for ABO and non-ABO antibody titres relative to tube testing. Vox Sang. 2020;115:314–22.
28. Kang SJ, Lim YA, Baik SY. Comparison of ABO antibody titers on the basis of the antibody detection method used. Ann Lab Med. 2014;34:300–6.
29. Lally K, Kruse RL, Smetana H, Davis R, Roots A, Marshall C, et al. Isohemagglutinin titering performed on an automated solid-phase and hemagglutinin-based analyzer is comparable to results obtained by manual gel testing. Transfusion. 2020;60:628–36.
30. Shim H, Hwang JH, Kang SJ, Seo HS, Park EY, Park KU, et al. Comparison of ABO isoagglutinin titres by three different methods: tube haemagglutination, micro-column agglutination and automated immunohematology analyzer based on erythrocyte-magnetized technology. Vox Sang. 2020;115:233–40.
31. Manook M, Mumford L, Barnett ANR, Osei-Bordom D, Sandhu B, Veniard D, et al. For the many: permitting deceased donor kidney

transplantation across low-titre blood group antibodies can reduce wait times for blood group B recipients, and improve the overall number of 000 MM transplants—a multicentre observational cohort study. Transpl Int. 2019;32:431–42.
32. TSANZ. Clinical guidelines for transplantation from deceased donors. The Transplantation Society of Australia and New Zealand. 2020. https://tsanz.com.au/storage/documents/TSANZ_Clinical_Guidelines_Version-14.pdf. Accessed 8 Dec 2020.
33. Perry H, Bovin N, Henry S. A standardized kodecyte method to quantify ABO antibodies in undiluted plasma of patients before ABO-incompatible kidney transplantation. Transfusion. 2019;59:2131–40.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Kahlyar H, Roxby D, Badrick T, Vanniasinkam T. Challenges in antibody titration for ABO-incompatible renal transplantation. Vox Sang. 2021;1–10.

Received: 31 January 2021 | Revised: 19 April 2021 | Accepted: 29 April 2021

DOI: 10.1111/vox.13131

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Passive immune basophil activation test for the identification of allergic episodes from various adverse events elicited by haematopoietic cell transplantation: A pilot study

Kazuta Yasui[1] | Terukazu Enami[2] | Ikue Okamura-Shiki[2] | Tomoaki Ueda[3] | Kentaro Fukushima[3] | Nobuki Matsuyama[1] | Takafumi Kimura[1] | Minoko Takanashi[4] | Naoki Hosen[3] | Takashi Ikeda[2] | Yoshihiro Takihara[1] | Fumiya Hirayama[1]

[1]Research and Development, Japanese Red Cross Kinki Block Blood Center, Ibaraki, Japan

[2]Division of Hematology and Stem Cell Transplantation, Shizuoka Cancer Center, Nagaizumi-chou, Japan

[3]Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Suita, Japan

[4]Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan

Correspondence
Kazuta Yasui, Research and Development, Japanese Red Cross Kinki Block Blood Center, 7-5-17 Saitoasagi, Ibaraki, Osaka 567-0085, Japan.
Email: kazuta-yasui@kk.bbc.jrc.or.jp

Abstract

Background and Objectives: Haematopoietic cell transplantation (HCT) therapy tends to be associated with various complications including engraftment failure, regimen-related toxicities, and infectious diseases. In addition, HC infusion itself occasionally elicits adverse events (AEs), one of the most common AEs is an allergic reaction. As appropriate laboratory tests have not yet been established to distinguish allergy-mediated AEs from other complications, clinical responses for HCT-related AEs can only be nonspecific. In this pilot study, using passive immune basophil activation test (pi-BAT), we attempted to distinguish an HC infusion-induced allergic reaction from various HCT-related AEs.

Materials and Methods: Using pi-BAT, we examined 34 patients who underwent HCT, that is, 11 with AEs and 23 without AEs as controls.

Results: Two of the eleven AE cases were pi-BAT positive and, the rest of nine AE cases were negative, while all non-AE cases were negative. Both of the two positive cases showed erythema, tachycardia, plus cough. Because erythema is one of the representative symptom of allergy, those cases could be classified as allergic reaction cases or anaphylaxis cases if tachycardia and cough were concomitant symptoms of erythema. Among the nine AEs with pi-BAT negative result, four cases showed urticaria, four showed vomiting plus diarrhoea, and one showed cough. Urticaria case was strongly suspected of allergy, however, the AE cases were pi-BAT negative.

Conclusion: The pi-BAT may be useful as an auxiliary diagnostic tool to confirm the possible involvement of HC infusion in HCT-related AEs and identify an immunologic mechanism for HCT-related hypersensitivity reactions.

KEYWORDS
allergic-adverse events, haematopoietic cell transplantation-related adverse events, passive immune basophil activation test

INTRODUCTION

Haematopoietic cell transplantation (HCT) of the bone marrow (BM), peripheral blood (PB), and cord blood (CB) cells is performed for the treatment of life-threatening haematopoietic malignancies as well as for immunological and congenital diseases. However, HCT is frequently associated with severe complications; high-dose chemotherapy and whole body irradiation used to ablate recipients' haematopoietic and immune cells lead to regimen-related toxicities affecting the lungs, kidneys, cardiovascular system, gastrointestinal system, and various other organs [1, 2]. Myelo- and immune-suppression result in bacterial, fungal, and viral infections. In addition, infusion of HC-product, including both cellular and plasma components, by itself can lead to adverse events (AEs) such as nausea/vomiting, flushing, allergic reactions, hypotension, hypertension, arrhythmia, and respiratory distress [3–11]. Although it is important to diagnose allergic diseases by clinical symptoms even in HC infusion-related AEs, that is not always easy. These HC-product infusion-related AEs coincide with the regimen-related AEs. Moreover, regimen-related AEs can augment or modify HC-product infusion-related AEs. In addition, different kinds of medication, such as antibiotics and non-steroid anti-inflammatory drugs, can be administered to patients just before or after HC-product infusion. Therefore, it is difficult to distinguish whether the AE episode is related to HC-product infusion by clinical symptoms and the timing of the onset of the symptoms. This has hampered our understanding of the causal relationship between HC-product infusion and AEs that occurred during or soon after HC-product infusion. Another difficulty in understanding HC-product infusion-related AEs is that compared with blood products, HC-product comprised various elements including the diversity, maturity, and viability of cellular lineages. Further, donor plasma including cytokines, such as G-CSF, are used for haematopoietic stem cell mobilization in the peripheral blood, and chemical substances such as dimethyl sulfoxide (DMSO) and dextran are used for cryopreservation of cord blood stem cells and peripheral blood stem cells [3, 12–15]. This is also the case with HC infusion-related allergic-AEs, it is important to determine causal relationship between HC infusion and elicited allergic reaction, and to demonstrate immunological mechanisms. In this regard, a helpful auxiliary test is necessary.

In contrast, blood cell products consist of simpler elements, and blood transfusion-associated AEs have been routinely and systemically surveyed through haemovigilance programs in many countries [16, 17]. In addition, recent studies have revealed a causal relationship between transfusion and transfusion-associated allergic-AEs and their underlying mechanisms in a substantial number of cases [18, 19]. The basophil activation test (BAT) and passive immune BAT (pi-BAT) have been introduced as auxiliary tests for investigating the causal relationship and mechanisms of transfusion-related allergic AEs, especially in Japan [20, 21]. In BAT, a patient's whole blood sample is incubated with the supernatant of residual blood products, and the subsequent basophil activation is assessed using flow cytometry based on up-regulation of cell degranulation/activation markers, such as CD63 and CD203c. If the test is positive, the causal relationship is strongly suggested. More specifically, to provide a detailed description about the utility of BAT and pi-BAT in transfusion medicine, we previously performed the BAT in two cases of severe anaphylaxis occurring immediately after PC transfusion [19]. Both cases tested positive, and the basophil activation was completely inhibited by treatment with dasatinib, a Bcr-Abl and Src family tyrosine kinase inhibitor that blocks several FcεRI downstream targets, thereby demonstrating IgE-dependent pathway. Subsequently, we have accumulated 11 more BAT-positive transfusion related allergic-AE cases, in all of which basophil activation was cancelled by dasatinib treatment (six cases reported in Ref. [18] and five unpublished cases). However, it cannot necessarily be performed for patients receiving therapy for myelosuppression caused by primary diseases and treatments because sufficient basophils for BAT is difficult to sample from these patients. pi-BAT was developed to remedy this limitation of BAT [20, 21]. In pi-BAT, basophils from healthy volunteers, whose own receptor-bound IgE is replaced with IgE of patients with transfusion-related allergic-AEs are activated by supernatants of the corresponding transfused blood products, that is, pi-BAT depends only on the patients' IgE and not on basophils. Regarding this assay system, some decades ago, Ishizaka and his colleagues conducted a study in which monkey lung fragments were passively sensitized with IgE antibodies from human serum; the release of histamines and leukotrienes was assessed upon challenge with either a specific allergen or an anti-IgE antibody [22]. A decade later, Pruzansky et al. modified this in vivo system to develop an in vitro system, in which basophils from healthy volunteers, whose own receptor-bound IgE was replaced by patient IgE, were activated by allergens to which the patients were sensitized, and applied this technology to the field of allergic diseases [23], and we applied this system to the field of transfusion medicine [21]. This is the background how pi-BAT was developed. The positive results of pi-BAT indicated causative relationship between transfusion and transfusion related allergic-AEs and theoretically suggested IgE dependent pathway. We observed that nine of 10 cases with moderate-to-severe transfusion related allergic-AE tested positive [24]. Additionally, we have reported three more pi-BAT positive transfusion mediated allergic AE cases [20], in one of which pretreatment with dasatinib completely inhibited basophil activation, clearly demonstrating IgE dependent pathway. Of note, there were no BAT positive nor pi-BAT positive cases in which disatinib failed to cancel basophil activation. Therefore, in the pi-BAT positive cases of this study the allergic clinical symptoms were suggested to be elicited via IgE dependent pathway. In addition, one group use a similar test to investigate transfusion-related allergic-AEs, in which cord blood derived-cultured mast cells (CBMCs) were first sensitized with IgE in the plasma of AE patient to mimic mast cells of the patient and then stimulated by corresponding blood product [25, 26]. Those CBMCs, similar to the basophils in our pi-BAT system, were activated via allergen/patient IgE pathway.

HC-product infusion-related allergic-AEs are reported to be symptomatic in 2%–7% of all HC-product infusion-related AEs [10, 11], although regimen-related and drug-induced AE cases might be possibly included. Considering the abovementioned current clinical

surroundings, further studies are needed to evaluate the causative relationship of allergic-AEs with HC-product infusion. We examined 34 patients who underwent HCT, 11 patients developed AEs and 23 patients did not develop AEs. Thereby, we here hypothesize that pi-BAT may be useful to detect HSC-product infusion-related allergic-AEs.

MATERIALS AND METHODS

Research ethics

This study was approved by the institutional review board of the Ethics Committee of the Japanese Red Cross Society, Blood Service Headquarters (2017-032-2). We obtained written informed consent from patients and healthy volunteers.

Clinical samples

Thirty-four patients who underwent HST at the Shizuoka Cancer Center and Osaka University Hospital between 2017 and 2020 were enrolled in this study. Eleven of 34 patients developed some AE, including nausea/vomiting, diarrhoea, flushing, hypotension, hypertension, arrhythmia, and respiratory distress, within 24 h after HC-product infusion, whereas no AEs were observed in the rest of 23 patients. The detailed information on these patients, including sex, age, underlying diseases, source of HCs, elicited symptoms, and elapsed time until onset is summarized in Table 1.

Peripheral whole blood was collected from the patients for the preparation of plasma samples; whole blood treated with ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant was obtained on the day before HC-product infusion from 33 patients, whereas 1 month before HC-product infusion from one patient (A11). Plasma was separated from the whole blood via centrifugation at 3000*g* for 10 min at 4°C and stored at −80°C. A small amount (approximately 100–200 μl) of residual HC-products was recovered at the medical institution immediately after HC-product infusion, except for A11, and the supernatants were separated via centrifugation at 3000*g* for 10 min at 4°C and stored at −80°C. In case of A11, the supernatant was separated from the frozen sample and stored for a later investigation.

Basophil sources and control IgE sources from healthy volunteers

Basophils were obtained from an EDTA-treated whole blood sample that was collected from a healthy laboratory volunteer in our blood centre. To separate mononuclear cells from the whole blood, 6% (w/v) hydroxyethyl starch 40 (HES40) was added at a final concentration of 1.2%. A mononuclear cell-rich supernatant was then separated via centrifugation at 70*g* for 5 min at 4°C to enhance red blood cell sedimentation. Peripheral blood mononuclear cells (PBMCs) were finally isolated from the mononuclear cell-rich supernatant by centrifugation at 200*g* for 5 min at 4°C. The PBMCs were used as the source of basophils in the activation tests described latter.

Control IgE were obtained from plasma samples that were collected and separated from 34 healthy volunteers, as described in "Clinical samples."

Reagents

Purified and PE-conjugated mouse anti-human IgE (AHIgE) monoclonal antibodies (MoAbs) were obtained from Beckman Coulter (Fullerton, CA). FITC-conjugated mouse anti-HLA-DR and PE-conjugated mouse anti-CD203c MoAbs were also purchased from Beckman Coulter. PE-Cy5-conjugated mouse anti-CD123 MoAb and FACS lysis solution were purchased from BD Biosciences (San Jose, CA). HES40 was purchased from NIPRO, Osaka Japan. Heparin sodium and $CaCl_2$ were purchased from Mochida Pharmaceutical Co. ltd., Tokyo, Japan, and Nacalai Tesque, Kyoto, Japan, respectively.

Passive immune BAT (pi-BAT)

Replacement of IgE bound on basophils was performed using the two-step procedure, as reported before [21]. Briefly, to remove receptor-bound IgE, 5×10^6 healthy volunteer PBMCs were suspended in 1 ml of lactic acid solution (13.4 mM lactate, 140 mM NaCl, and 5 mM KCl) and incubated for 5 min on ice. To stop the reaction, the PBMC suspension was neutralized by the addition of 1 ml human serum albumin (HSA) solution (RPMI-1640 medium [pH 8.0] supplemented with 0.5% HSA and 12 mM Tris–HCl). The cell suspension was centrifuged at 1200*g* for 5 min at 4°C. After washing with PBS, the cell pellet was resuspended in 0.5 ml of the plasma under examination supplemented with 10 U/ml heparin sodium and 10 mM $CaCl_2$ and incubated for 60 min at 37°C. Plasma as a source of IgE was derived from either healthy volunteers (control IgE) or patients before HSC-product infusion (test IgE). We defined basophils in the cell pellet, whose own receptor-bound IgE was replaced by third party IgE (control or test IgE), as "quasi-basophils." Fifty microliter of the cell suspension containing quasi-basophils was incubated with 5 μL of PBS or AHIgE (5 μg/ml). In case of the quasi-basophils with test IgE, the cell suspension was incubated with 5 μl of not only PBS or AHIgE but also the supernatants of relevant residual HC products. After incubation, the mixture was added to 200 μl of PBS containing 1% BSA (Sigma Chemical, MO) and 2 mM EDTA (PBS/BSA/EDTA) and centrifuged at 200 for 2 min. The cells were then incubated with a cocktail of monoclonal antibodies against HLA-DR, CD123, and CD203c for 15 min at 4°C and washed with PBS/BSA/EDTA. After removing RBCs using the FACS lysis solution, the cells were subjected to flow cytometric analysis. HLA-DR-negative and CD123-positive cells in the forward scatter versus side scatter blast cell window were identified as basophils. CD203c was used as a basophil activation marker. The

TABLE 1 Clinical characterization of patients and pi-BAT results

Patient					Adverse event		
Sample number[a]	Age	Sex[b]	Primary disease	Infused HC source[c]	Elicited symptoms	Elapsed time until onset	pi-BAT[d]
N1	46	M	Plasma cell leukaemia	Allogenic PBMC	None	–	–
N2	28	M	Acute lymphoid leukaemia	Allogenic BM	None	–	–
N3	37	M	Acute T-cell leukaemia	CB	None	–	–
N4	42	M	Acute myeloid leukaemia	Allogenic PBMC	None	–	–
N5	66	M	Acute myeloid leukaemia	Allogenic PBMC	None	–	–
N6	59	M	Acute myeloid leukaemia	CB	None	–	–
N7	57	F	Follicular lymphoma	Allogenic BM	None	–	–
N8	51	M	Adult T-cell leukaemia	Allogenic PBMC	None	–	–
N9	70	F	Acute myeloid leukaemia	CB	None	–	–
N10	59	M	Richter Syndrome	Allogenic BM	None	–	–
N11	63	M	Acute myeloid leukaemia	CB	None	–	–
N12	50	M	Acute myeloid leukaemia	CB	None	–	–
N13	56	F	Myelodysplastic syndromes	Allogenic PBMC	None	–	–
N14	57	M	Myelodysplastic syndromes	Allogenic BM	None	–	–
N15	22	M	Acute myeloid leukaemia	CB	None	–	–
N16	48	F	Acute B-cell leukaemia (Ph negative)	Allogenic BM	None	–	–
N17	68	M	Myelodysplastic syndromes	Allogenic BM	None	–	–
N18	24	M	Blastic crisis of chronic myeloid leukaemia	Allogenic BM	None	–	–
N19	63	M	Acute myeloid leukaemia with myelodysplasia-related changes	Allogenic BM	None	–	–
N20	44	M	Anaplastic large cell lymphoma, ALK-positive	Allogenic PBMC	None	–	–
N21	58	M	Anaplastic large cell lymphoma	CB	None	–	–
N22	22	M	Acute myeloid leukaemia	CB	None	–	–
N23	32	M	Acute T-cell leukaemia	CB	None	–	–
A1	49	F	Chronic myeloid leukaemia	Allogenic PBMC	Vomiting, diarrhoea	12 h	–
A2	44	F	Diffuse large B-cell lymphoma	CB	Vomiting, diarrhoea	24 h	–
A3	43	F	Myelodysplastic syndromes	Allogenic PBMC	Vomiting, diarrhoea	6 h	–
A4	37	F	Acute lyphoid leukaemia	CB	Vomiting, diarrhoea	2 h	–
A5	59	M	Acute lyphoid leukaemia	Allogenic BM	Cough (intermittent)	3 h	–
A6	51	F	Acute lyphoid leukaemia	Allogenic BM	Urticaria (partially)	30 min	–
A7	68	F	Acute myeloid leukaemia	allogenic BM	Urticaria (partially)	1 h	–
A8	46	M	Primary myelofibrosis	Allogenic PBMC	Urticaria (partially)	10 min	–
A9	51	F	Acute lyphoid leukaemia	Allogenic BM	Urticaria (partially)	2 h	–
A10	35	F	Acute myeloid leukaemia	Allogenic BM	Erythema, tachycardia, cough (intermittent)	2 h	+
A11	42	F	Acute myeloid leukaemia	CB	Erythema, tachycardia, cough (intermittent)	10 min	+

[a]N and A show "non-AE" and "AE."
[b]M and F show male and female.
[c]BM, CB, and PBMC are an abbreviation for bone marrow, cord blood, and mobilized peripheral blood mononuclear cell, respectively.
[d]+ and − show positive and negative.

cursor was adjusted on the CD203c histograms at the point where 5% of the basophils were CD203c-positive without stimulation to calculate the percentage of CD203c-positive basophils.

RESULTS

A schematic preparation procedure of quasi-basophils through the two-step procedure, pretreatment, and functional assessment is shown in Figure 1a. To confirm that IgE from own basophils was replaced with that of independent individuals, we measured surface IgE expression during each step using flow cytometry (FCM). As shown in Figure 1b, treatment with lactic acid dissociated the majority of the surface IgEs, and treatment with plasma from the independent individuals completely reconstituted the cell-bound IgE. Before stimulating with residual HC-products, which can contain some allergen(s), we further examined whether those quasi-basophils can transmit an activation signal. Quasi-basophils treated with IgEs from 34 independent

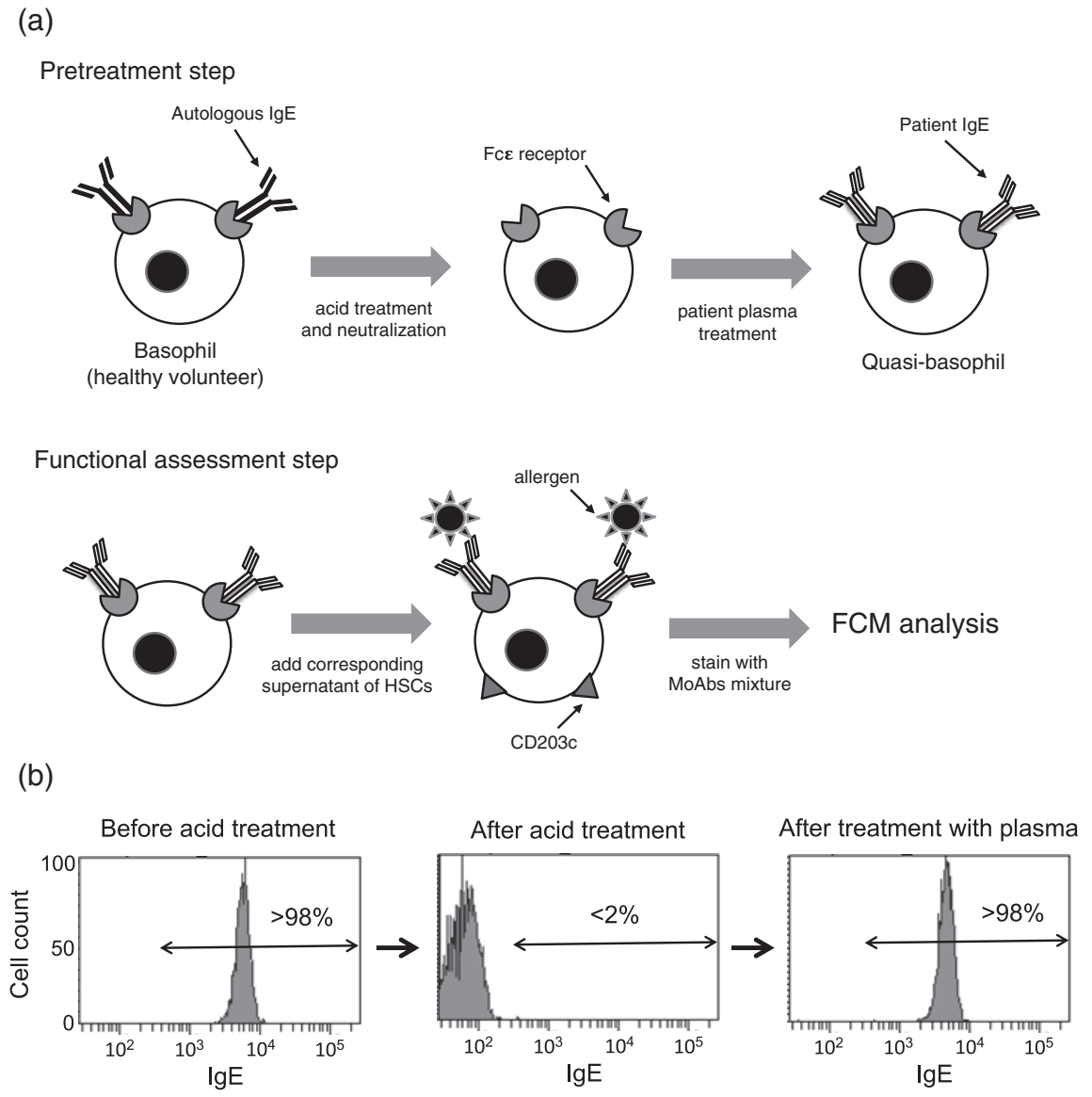


FIGURE 1 Implementation process of passive immune basophil activation test (pi-BAT). (a) pi-BAT consists of two processes: pretreatment and function process. In the pretreatment process, IgE of basophils' own origin is replaced with that of the third party's origin by lactic acid treatment and the subsequent treatment with independent individual plasma. In the function assessment process, the activation of basophils is mediated via allergen/IgE-dependent pathway. The allergen/IgE-dependent pathway occurs after high-affinity FcεRI aggregates in response to the cell surface-binding of IgE in complexes with specific allergens. (b) Replacement of receptor-bound IgE after treatment with lactic acid and independent individual plasma is shown. Peripheral blood mononuclear cell samples are stained with anti-HLA-DR, anti-CD123, and anti-human IgE monoclonal antibodies. HLA-DR-negative CD123-positive cells in the forward scatter versus side scatter blast cell window are identified as basophils, and human IgE expression is determined. Expression of cell surface IgE on basophils after each step is presented in the following panels: pretreated basophils, acid-treated basophils, and quasi-basophils

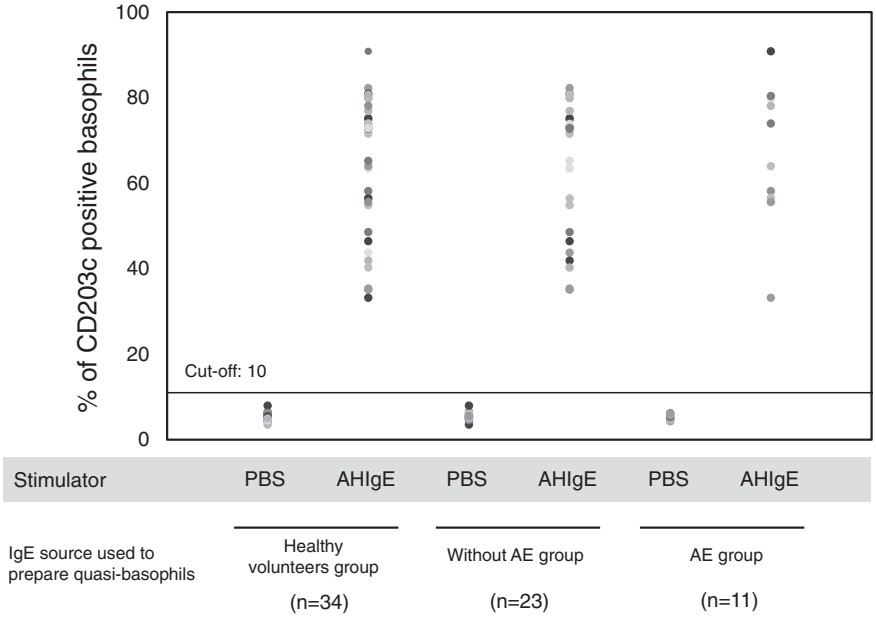


FIGURE 2 Activation of quasi-basophils. Quasi-basophils stimulated with AHIgE as the positive control and PBS as the negative control; activation of quasi-basophils measured using the expression levels of CD203c. The activation of quasi-basophils in which their own surface IgE is replaced with IgEs of 34 healthy volunteers (healthy volunteers group), 23 patients without AE, or 11 patients with AEs is shown. The percentage of CD203c-positive quasi-basophils exceeding 10% (solid line) is categorized as pi-BAT positive

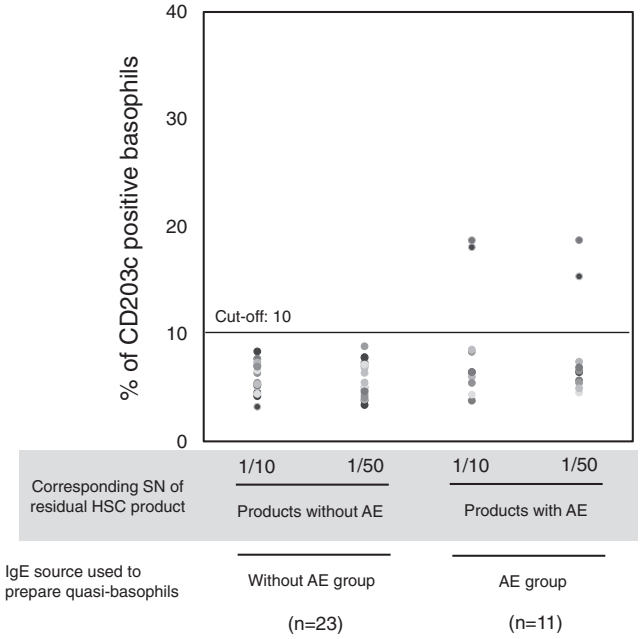


FIGURE 3 Activation of quasi-basophils pretreated with patients' IgE by the corresponding supernatant of residual HC products. Quasi-basophils pretreated with patients' IgE are stimulated with the corresponding supernatant (SN) of residual HC product (1/10 and 1/50 dilution). The activation of quasi-basophils in which the own IgE surface is replaced with IgEs of 23 patients without AEs or IgEs of 11 patients with AEs (the AE group) is shown

healthy volunteers, 23 patients without AE, or 11 patients with AEs were stimulated by PBS as a negative control or an AHIgE as a positive control. As shown in Figure 2, in all three groups, the background response levels elicited with PBS were quite low, whereas the maximum responses elicited by AHIgE were significantly high, stating that we can easily distinguish the maximum response levels from the background ones. We therefore adjusted the cutoff point to 10% in this study.

Having demonstrated that quasi-basophils were eligible for subsequent activation experiments, we further investigated their response to the corresponding supernatants from residual HC-products, a part of the transplanted product. When quasi-basophils pretreated with plasma from 23 patients without AE were examined as the control group (Figure 3-left) or 11 patients with AE as the test group (Figure 3-right) were stimulated with the corresponding supernatant of residual HC-products, the quasi-basophils of 23 without AE and nine of 11 with AE tested negative in pi-BAT. In contrast, two patients (A10 and A11) with AE showed significantly higher responses and were classified as positive in pi-BAT.

Some HC-product infusion-related allergic-AEs can be caused by DMSO and dextran used during the cryopreservation processes of HC-products [4, 6–8, 15]. The HC sources of cases A10 and A11 were fresh BM and cryopreserved CB, respectively, and the latter contained DMSO and dextran. To explore the possibility that DMSO and dextran worked as allergen(s) in case A11, these molecules were added in place of the supernatant of residual HC-product of case A11 to stimulate quasi-basophils treated with case A11's patient's IgE. DMSO and dextran, both at 1/10 or 1/100 dilution, yielded negative pi-BAT results, stating that both DMSO and dextran can hardly be an allergen in case A11 (data not shown).

DISCUSSION

Among 11 cases diagnosed with HC-product infusion-related AE, two cases (A10 and A11) were shown to be positive in pi-BAT, whereas the remaining nine cases with AEs nor 23 control cases without HC-product infusion-related AEs were shown to be negative. Therefore, in the two positive cases, it was strongly suggested that elicited by HC infusion via the IgE-dependent pathway. However, the allergen was not determined. It was also revealed that the specificity of pi-BAT calculated using cases "without the AE, as negative control sample" was as high as 1.0 (23/23), whereas the sensitivity could not be calculated because there was no way to verify which case was allergic. Instead, pi-BAT determined some HC-product infusion-related-allergic episodes from various AEs elicited by HCT. We examined only 11 cases of HC-product infusion-related AEs, therefore, similar but larger studies are required to conclusively evaluate the usefulness of pi-BAT.

The remaining nine cases with AEs were pi-BAT negative; there can be one possibility. Four urticaria cases, A6 to A9, were unexpectedly pi-BAT negative. In our previous study using transfusion related allergic-AE samples, the sensitivity of pi-BAT to detect mild allergic transfusion reactions was much lower than that to detect moderate-to-severe allergic reactions. The pi-BAT, therefore, may have not detected the four urticaria cases. In contrast, the two pi-BAT positive cases showed erythema, tachycardia, plus cough. Because erythema is a representative symptom of allergy, we believe that these two were allergic cases. If tachycardia and cough were concomitant symptoms of the erythema, the two cases could be classified as anaphylaxis. In the two pi-BAT-positive cases, the reactivity of the quasi-basophils stimulated with the corresponding supernatants of residual HC-products was lower than that with AHIgE. The AHIgE used as a positive control could transduce activation signals through all IgE/Fcε receptor complex in quasi-basophils, whereas the supernatants of HC-product can transduce only via Fcε receptors grasping the corresponding specific IgE, which is considered as only a small part of total Fcε receptors. This can be one of the reasons for the difference in the magnitude of basophil activation between the positive control and the corresponding supernatants of residual HC-products.

In the two cases with positive pi-BAT, we were unable to identify the responsible antigen(s) presumed to be present in the causative HC-products. DMSO and dextran were unlikely to be responsible in both cases. Even in blood transfusion-related-allergic AEs, candidate allergens have been suggested only in a few cases; haptoglobin, a plasma protein, was responsible in a case of anaphylactic shock in a homozygous haptoglobin-deficient patient [27], and methylene blue, which was used to inactivate the pathogen of plasma products, was responsible in three cases of anaphylactic shock [28, 29]. In addition, peanut allergen was recently identified as an allergen in a case of transfusion-associated anaphylaxis [30]. Therefore, the identification of candidate allergens is a challenge for the future.

The pi-BAT is an auxiliary tool for identification of causative relationship between allergic-AE and HC infusion. In this regard, it is superior to tryptase test, which cannot explore the issue of causative relationship. The importance of specific IgE test is quite limited in investigation of HC infusion related AE cases because of no allergens having been identified with some exceptions, such as dextran and DMSO. Although dextran and DMSO have been suspected as allergen, there has been no definitive evidence reported and there is no commercial test kit for detecting IgE antibodies against them. In addition, the pi-BAT utilize patient plasma (serum) rather than patient basophils, therefore, there is no need to perform the test immediately after the onset of AE, if patient plasma before HCT is available. Considering the time grace, we believe that it is better to carry out the pi-BAT in appropriate reference laboratories rather than in individual medical institutions.

CD63 and CD203c are representative surface markers for the identification of activated basophils. Although surface expression of CD63 corresponds to the early stage of basophil activation, it is also an activation marker for platelets. Platelet activation leads to the production of platelet microparticles with upregulation of P-selectin and CD63 expression on their surface. These activated platelets and microparticles may bind to basophils via PSGL-1, a ligand of P-selectin, leading to a false-positive result. In contrast, CD203c is expressed only by basophils and their precursors. Additionally, in our previous studies, we observed that CD63 was a less sensitive marker compared with CD203c [21]. Therefore, we believe that CD203c is a better marker for basophil activation compared with CD63.

The conditioning regimen-related toxicities frequently occur shortly after HC-product infusion, and it is difficult to distinguish such conditioning regimen-related toxicities and HC-product infusion-related AEs [10]. HC-product infusion-related AEs have been clinically diagnosed on the basis of the symptoms, the timing of onset, and the absence of other possible causal factors such as medication. Therefore, to precisely diagnose infusion-related AEs, it is necessary to develop a sensitive enough diagnostic system to distinguish infusion-related AEs. In this regard, pi-BAT can serve as a useful system. pi-BAT can also refer to a causal relationship between the allergic AE and HC-product infusion in the cases with positive pi-BAT findings. In addition, pi-BAT can serve as an auxiliary diagnostic tool in HC-product infusion-related AEs, where it is difficult to conclude whether it is an allergy only by symptoms. In particular, when a patient developed serious transfusion-related-allergic AEs due to blood transfusions before HC-product transplantation, the patient may also have possibility to develop severe allergic-AE by HSC-product infusion. For such patients, it may be useful to pre-test the reactivity between patient own IgE and plasma component of HC-products to be transplant using the pi-BAT. If the pi-BAT shows positive result, the patient may develop HC-product infusion-related allergic-AEs. In such cases it would be recommendable to wash the HC-products. Considering that most transfusion-related allergic AEs are prevented by washing the blood products before transfusion, washing HC-products may avoid HC-product infusion-related allergic-AEs, although substantial numbers of HC may be lost in the washing procedure. Although this was a primary study to elucidate the causal relationship between allergic AE and HC-product infusion, it may provide an important clue for further detailing molecular and cellular mechanisms underlying allergic AEs in the future.

ACKNOWLEDGEMENTS

We thank Dr Mitsunobu Tanaka, Dr Ayumu Kuroishi, Ms Yoriko Irie, and Mr Akihiro Fuchisaki for their assistance in preparing this manuscript. We also thank Enago (https://www.enago.jp) for English language editing.

KY designed and performed experiments, discussed results, and wrote the paper; YT discussed results and edited the paper; NM performed experiments; TE, IS, TU, KF, MT, NH, and TI discussed results; TK discussed results and edited the paper; and FH contributed to experimental design, discussed results, and edited the paper.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript.

ORCID

Kazuta Yasui https://orcid.org/0000-0001-8714-2949
Fumiya Hirayama https://orcid.org/0000-0001-6518-2085

REFERENCES

1. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006;354:1813–26.
2. Diaconescu R, Flowers CR, Storer B, Horror ML, Maris MB, Maloney D, et al. Morbidity and mortality with Nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. Blood. 2005;104:1550–8.
3. Shu Z, Heimfeld S, Gao D. Hematopoietic SCT with cryopreserved grafts: adverse reactions after transplantation and cryoprotectant removal before infusion. Bone Marrow Transplant. 2014;49:469–76.
4. Benekli M, Anderson B, Wentling D, Bernstein S, Czuczman M, McCarthy P. Severe respiratory depression after dimethylsulphoxide-containing autologous stem cell infusion in a patient with AL amyloidosis. Bone Marrow Transplant. 2000;44:907–16.
5. Sauer-Heilborn A, Kadidlo D, McCullough J. Patient care during infusion of hematopoietic progenitor cells. Transfusion. 2004;44:907–16.
6. Junior AM, Arrais CA, Saboya R, Velasques RD, Junqueira PL, Dulley FL. Neurotoxicity associated with dimethylsulfoxide-preserved hematopoietic progenitor cell infusion. Bone Marrow Transplant. 2008;41:95–6.
7. Ruiz-Delgado GJ, Mancias-Guerra C, Tamez-Gómez EL, Rodríguez-Romo LN, López-Otero A, Hernández-Arizpe A, et al. Dimethyl sulfoxide-induced toxicity in cord blood stem cell transplantation: report of three cases and review of the literature. Acta Haematol. 2009;122:1–5.
8. Gonzalez-Lopez TJ, Sanchez-Guijo FM, Ortin A, Crusoe E, Cordoba I, Corral M, et al. Ischemic stroke associated with the infusion of DMSO-cryopreserved auto-PBSCs. Bone Marrow Transplant. 2011;46:1035–6.
9. Hausmann A, Fischer N, Breitkopf S, Menne F, Jess K, Schmidmayr S, et al. Severe motor aphasia after reinfusion of cryopreserved autologous stem cells after myeloablative conditioning. Transfusion. 2016;56:2615–7.
10. Ikeda K, Ohto H, Okuyama Y, Yamada-Fujiwara M, Kanamori H, Fujiwara SI, et al. Adverse events associated with infusion of hematopietic stem cell products: a prospective and multicenter surveillance study. Transfus Med Rev. 2018;32:186–94.
11. Ikeda K, Ohto H, Yamada-Fujiwara M, Okuyama Y, Fujiwara S, Muroi K, et al. Hematopoietic cell infusion-related adverse events in pediatric/small recipients in a prospective/multicenter study. Transfusion. 2020;60:1015–23.
12. Calmens B, Lemarie C, Esterni B, Malugani C, Charbonnier A, Coso D, et al. Occurrence and severity of adverse events after autologous hematopoietic cell infusion are related to the amount of granulocytes in the apheresis product. Transfusion. 2007;47:1268–75.
13. Martín-Henao GA, Resano PM, Villegas JMS, Manero PP, Sánchez JM, Bosch MP, et al. Adverse reactions during transfusion of thawed haematopoietic progenitor cells from apheresis are closely related to the number of granulocyte cells in the leukapheresis product. Vox Sang. 2010;99:267–73.
14. Watz E, Remberger M, Ringden O, Ljungman P, Sundin M, Mattsson J, et al. Quality of the hematopoietic stem cell graft affects the clinical outcome of allogenic stem cell transplantation. Transfusion. 2015;57:1522–6.
15. Otrock ZK, Sempek DS, Carey S, Grossman BJ. Adverse events of cryopreserved hematopoietic stem cell infusion in adults: a single-center observational study. Transfusion. 2017;57:1997–2003.
16. Delanney M, Wendel S, Bercovitz RS, Cid J, Cohn C, Dunbar NM, et al. Transfusion reactions: prevention, diagnosis, and treatment. Lancet. 2016;388:2825–36.
17. Harvey AR, BasavaraJu SV, Chung KW, Kuehnert MJ. Transfusion-related adverse reactions reported to the National Healthcare Safety Network Hemovigilance Module, United States, 2010 to 2012. Transfusion. 2015;55:241–51.
18. Yasui K, Matsuyama N, Takihara Y, Hirayama F. New insights into allergic transfusion reactions and their causal relationships, pathogenesis, and prevention. Transfusion. 2020;60:1590–601.
19. Okamura-Shiki I, Matsuyama N, Yasui K, Hirayama F, Ikeda T. Clinical utility of the basophil activation test for analysis of allergic transfusion reactions: a pilot study. Vox Sang. 2017;112:114–21.
20. Hirayama F, Yasui K, Matsuyama N, Okamura-Shiki I. Possible utility of the basophil activation test for the analysis of mechanisms involved in allergic transfusion reactions. Transfus Med Rev. 2018;32:43–51.
21. Yasui K, Matsuyama N, Okamura-Shiki I, Ikeda T, Ishii K, Furuta RA, et al. Clinical utility of a passive immune basophil activation test for the analysis of allergic transfusion reactions. Transfusion. 2017;57:2084–95.
22. Ishizaka T, Ishizaka K, Orange RP, Austen KF. The capacity of human immunoglobulin E to mediate the release of histamine and slow reacting substance of anaphylaxis from monkey lung. J Immunol. 1970;104:335–43.
23. Pruzansky JJ, Grammer LC, Patterson R, Roberts M. Dissociation of IgE receptors on human basophils. I. Enhanced passive sensitization for histamine release. J Immunol. 1983;131:1949–53.
24. Yasui K, Matsuyama N, Kimura T, Kato H, Oka K, Imada K, et al. Sensitivity and specificity of passive immune-basophil activation test to detect allergic transfusion reactions. Transfusion. 2019;59:3308–13.
25. Abe T, Matsumoto C, Shimada E, Mazda T, Takanashi M, Kawaguchi K, et al. Immunoglobulin E oligomers identified in blood components activate mast cells: relevance to anaphylactic transfusion reaction. Transfusion. 2011;51:2327–36.
26. Abe T, Shimada E, Takamura T, Motoji K, Okazaki H. Antibody against immunoglobulin E contained in blood components as causative factor for anaphylactic transfusion reactions. Transfusion. 2014;54:1953–60.
27. Shimode N, Yasuoka H, Kinoshita M, Hirashima K, Tsujimoto S, Tashiro C, et al. Severe anaphylaxis after albumin infusion in a patient with ahaptoglobinemia. Anesthesiology. 2006;105:425–6.

28. Nubret K, Delhoume M, Orsel I, Laudy JS, Sellami M, Nathan N. Anaphylactic shock to fresh-frozen plasma inactivated with methylene blue. Transfusion. 2011;51:125–8.
29. Dewachter P, Castro S, Nicaise-Roland P, Chollet-Martin S, le Beller C, Lillo-le-Louet A, et al. Anaphylactic reaction after methylene blue-treated plasma transfusion. Br J Anaesth. 2011;106:687–9.
30. Anani W, Dobrozsi S, Punzalan R. Identification of peanut allergen in a transfused blood product causing transfusion associated anaphylaxis. Transfusion. 2020;60:1108–9.

How to cite this article: Yasui K, Enami T, Okamura-Shiki I, Ueda T, Fukushima K, Matsuyama N, et al. Passive immune basophil activation test for the identification of allergic episodes from various adverse events elicited by haematopoietic cell transplantation: A pilot study. Vox Sang. 2021;1–9.

Received: 1 February 2021 | Revised: 5 April 2021 | Accepted: 6 April 2021

DOI: 10.1111/vox.13119

SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

A case of Philadelphia chromosome-positive acute lymphocytic leukaemia with type I CD36 deficiency

Marina Matsui[1] | Koichi Kajiwara[1] | Masao Tsukada[1] | Kanichi Iwama[1] | Kazunari Yamada[1] | Hideki Kodo[1] | Katsuya Seto[2] | Kaoru Tonami[2] | Moe Ando[2] | Mika Matsuhashi[2] | Naoko Watanabe-Okochi[2] | Nelson Hirokazu Tsuno[2] | Yasuji Kozai[1]

[1]Department of Transfusion Medicine, Tokyo Metropolitan Tama Medical Center, Tokyo, Japan

[2]Laboratory Department, Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Tokyo, Japan

Correspondence
Marina Matsui, Department of Transfusion Medicine, Tokyo Metropolitan Tama Medical Center, 2-8-29 Musasidai, Fuchu, Tokyo 183-8524, Japan.
Email: hiramari0130@yahoo.co.jp

Abstract

Background: CD36 is a glycoprotein expressed on platelets and monocytes of the blood. There are two types of CD36 deficiency, type I and type II. Individuals with type I-deficiency do not express CD36 in any cell type and can produce the CD36 antibody, which causes pathological conditions, such as fetal/neonatal alloimmune thrombocytopenia (FNAIT) and platelet transfusion refractory (PTR), through antigenic exposure via transfusion or pregnancy.

Case presentation: We experienced a case of Philadelphia-positive acute lymphoblastic leukaemia with PTR. In addition to the CD36 antibody, multiple-specificity HLA antibodies were present in the patient's plasma, requiring transfusion of HLA-compatible and CD36-negative platelets (PC-HLA). Since the number of donors was limited, it was necessary to set-up a blood transfusion schedule so that hyper-fractionated cyclophosphamide, vincristine and doxorubicin therapy (hyper-CVAD) and ponatinib combination chemotherapy could be safely administered to achieve molecular remission. Rituximab administration resulted in reduced levels of both CD36 antibody and HLA antibody. Given the expression of CD36 on haematopoietic stem cells and the limited availability of CD36-negative PC-HLA, haematopoietic stem cell transplantation (HSCT) was not considered to be an option.

Conclusion: If CD36-negative, allogeneic haematopoietic stem cell donors are unable to be found, the indications for HSCT in patients with type I CD36-deficiency should be carefully weighed. In the present case, molecular remission has been able to be maintained to the present day after completion of a two-year maintenance regimen.

KEYWORDS
antibody-function, haematopoietic stem cell, patient blood management, refractoriness (platelets)

INTRODUCTION

CD36, also known as glycoprotein (GP)-IV or Nak-a, is normally expressed on the surface of platelets and monocytes and reportedly also on erythroid and myeloid cells of the blood [1–3]. There are two types of CD36 deficiency, namely type I, in which CD36 is absent on all cell types, and type II, in which CD36 is absent only on the surface of platelets. CD36-deficiency is found in 3%–11% of the Japanese

population, but type I-deficiency accounts for less than 10% of CD36-deficiency cases and has an incidence of 0.5%–1.0% [4, 5]. CD36 isoantibodies are produced by patients with type I-deficiency through exposure to CD36-positive cells via blood transfusion or pregnancy and can cause clinical conditions, such as platelet transfusion refractoriness (PTR) and fetal/neonatal alloimmune thrombocytopenia (FNAIT).

We herein present a case of Philadelphia chromosome-positive acute lymphocytic leukaemia (Ph + ALL) eventually diagnosed as type I CD36-deficiency. A high CD36 antibody titre and multiple-specificity HLA antibodies were identified in the patient's plasma. Locating PC donors based on HLA and CD36 compatibility was difficult and affected the choice of treatment modality, including haematopoietic stem cell transplantation (HSCT).

CASE REPORT

A 31-year-old female patient with a history of two pregnancies but no history of blood transfusion was referred to our hospital with the complaints of headache and palpitation. At admission, laboratory findings revealed leukocyte count 122 $\times$ 10^9/L, haemoglobin 3.2 g/dl, and platelet count 13 $\times$ 10^9/L indicating marked leukocytosis, severe anaemia, and thrombocytopenia. The differential leukocyte count revealed 96% lymphoblasts, which were positive for CD10, CD19, and CD20 and negative for CD7, CD2, and CD3 on flow-cytometry. Bone marrow aspiration resulted in a dry tap. Molecular genetic testing revealed positivity for minor BCR-ABL chimeric transcript, confirming the diagnosis of Ph + ALL. No ABL1 gene mutation (T315I) was detected. After admission, red cell concentrates (RCC), and platelet concentrates (PC) were transfused, and prednisolone administration (PSL, 60 mg/m^2) was initiated. After the fourth PC transfusion, however, the transfusion efficacy, as evaluated by the corrected count increment (CCI), was found to be poor. On hospitalization day 9, PTR was diagnosed, and the Japanese Red Cross Blood Center (JRCBC) was requested to provide tests for HLA antibodies and supplies of HLA-compatible PC.

Anti-platelet antibody testing and CD36 expression

The CD36 antibody and HLA antibodies with multiple specificities, including HLA-A24, and -B44, were identified in the patient's plasma. The CD36 antibody was detected using mixed-passive hemagglutination (MPHA) method (Beckman Coulter, Japan), a binding assay commercially available only in Japan, and PAKLx (Immucor, Waukesha, WI). The concomitant HLA antibodies in the patient's serum had a low-range specificity, and the target antigens in MPHA were pretreated with chloroquine to eliminate HLA reactivity. Thus, anti-CD36 antibody could be clearly identified, without affection by anti-HLA antibodies in MPHA. No other HPA antibodies were identified by the same method.

Flow cytometry using anti-CD36 monoclonal antibody was negative for CD36 antigen expression on both platelets and monocytes, confirming the diagnosis of type I-deficiency. Additionally, the patient's sibling (brother) was also tested and found to be CD36-positive (Figure 1).

Genetic testing

The exons of the code region of the CD36 gene in the genomic DNA extracted from the plasma were sequenced. The patient was found to be harbouring compound heterozygous mutations of c.329_330delAC at exon 5 and c.949insA at exon 10.

Clinical course

Supply of compatible PC

HLA-compatible, CD36-positive PC (10 units/bag containing 2–3 $\times$ 10^{11} platelets) was transfused on hospitalization day 14, but the platelet count was not able to be increased. On day 16, HLA-compatible, CD36-negative PC (10 units) was transfuse, resulting in an elevation of the platelet count from 6 $\times$ 10^9/L to 48 $\times$ 10^9/L (24 h CCI of 27,300/ml). To obtain supplies of HLA-compatible, CD36-negative PC, the JRCBC was required to locate compatible donors in their registry, schedule the apheresis collection, and perform compatibility testing. Therefore, working closely with the JRCBC to establish a schedule for the PC transfusion was necessary before initiating chemotherapy.

Evaluating antibodies in the patient's plasma

Flow cytometry using the indirect fluorescent antibody test revealed a high CD36 antibody titre (highest titre: 2048 times). Weekly administration of rituximab (375 mg/m^2) for four cycles aimed at reducing the CD36- and HLA-antibody titers was begun on day 19. Also, after day 15, CD36-positive PC was avoided, and only CD36-negative PC was transfused. After four months, the CD36-antibody titre decreased to 1:64, and antibodies to HLA-A24 and -B44 became undetectable (Figure 2).

Treatment of Ph + ALL

Following the initial administration of prednisolone, dasatinib (100 mg) was begun on day 11 but was discontinued due to persistent leukopenia, and ponatinib (30 mg) was begun on day 55. After the supply schedule for HLA-compatible, CD36-negative PC was confirmed, hyper-CVAD was begun on day 62. The patient achieved molecular remission after one cycle of hyper-CVAD, and ponatinib was reduced to 15 mg. HSCT was not indicated for the following reasons: (1) the sibling (brother) was HLA-incompatible and CD36-positive; (2) the Japan Marrow Donor Program (JMDP) and the

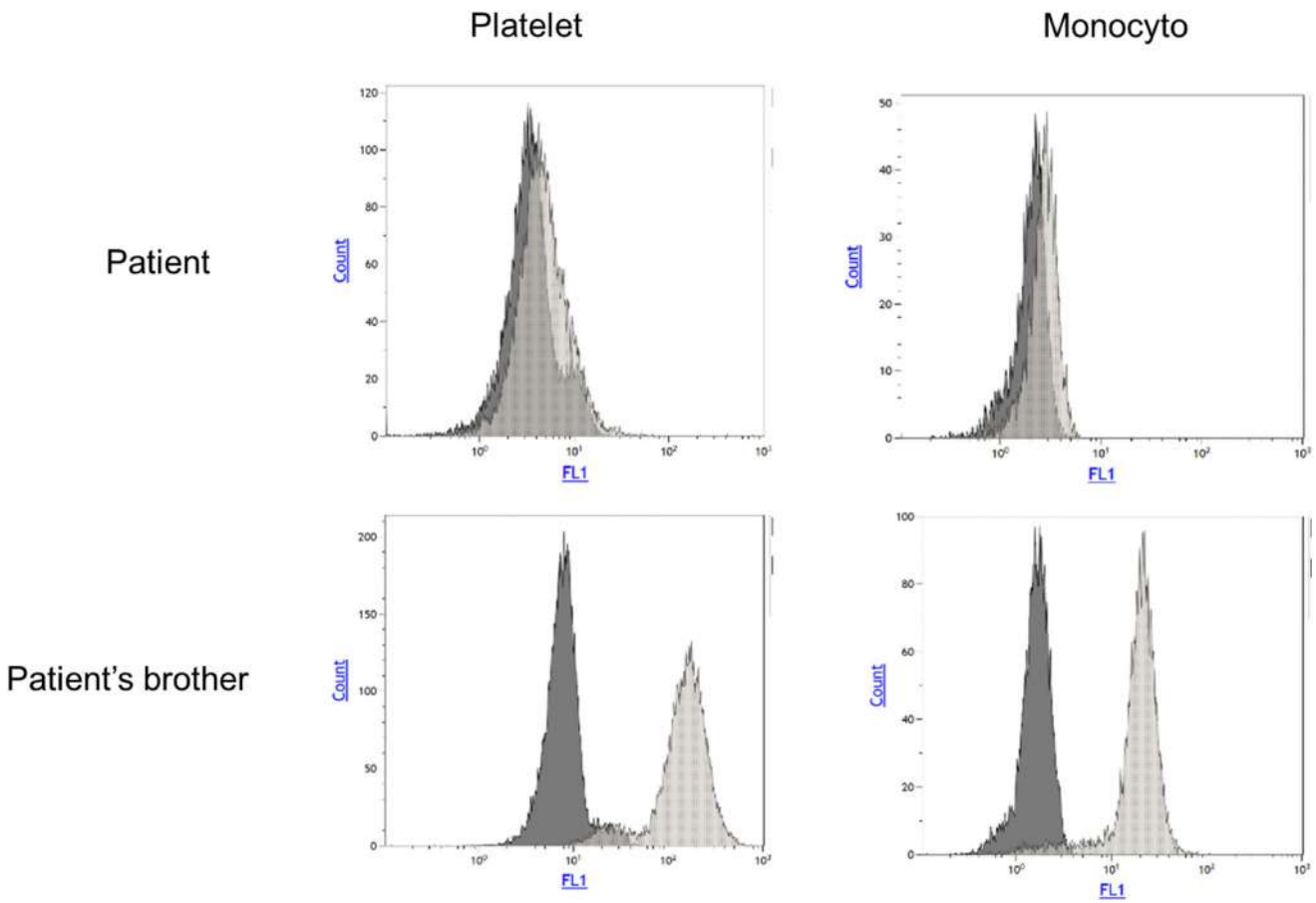
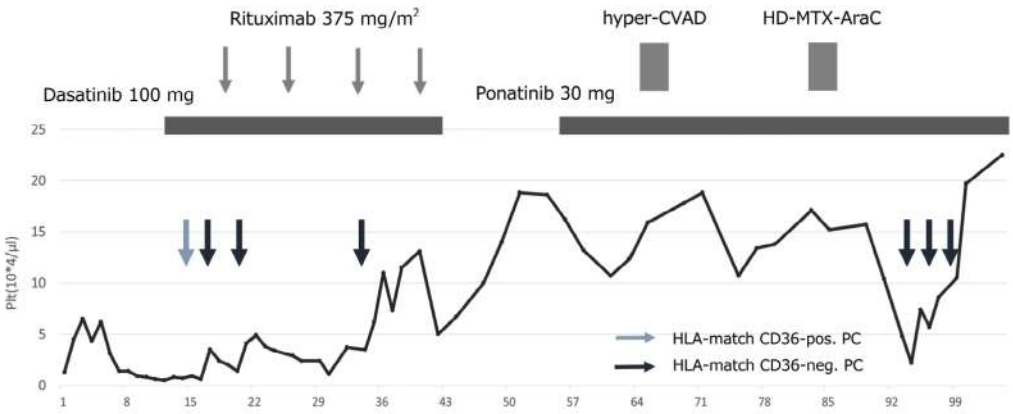


FIGURE 1 Flow cytometry of CD36 expression on platelets and monocytes using anti-CD36 antibody (FA6.152, Beckman coulter). The patient's platelets and monocytes were negative for CD36 expression while those of her sibling were positive (the dark grey histogram represents the negative control) [Colour figure can be viewed at wileyonlinelibrary.com]



CD36-antibody		×4	×2048	×512	×512	×512	×256	×128	×64
HLA-antibody (n-MFI)	A24	271	14210	3465	8133	5036	5333	2098	858
	B44	58	4779	100	1879	1009	1162	437	217

FIGURE 2 Patient's clinical course and changes in anti-CD36 and anti-HLA antibody levels. The CD36 antibody titre was measured by flow cytometry using the indirect fluorescent antibody test. Platelets from CD36-positive volunteer donors were used for antibody titration. n-MFI (normalized mean fluorescent intensity) of the main HLA antibodies identified by the Luminex assay are shown, with n-MFI < 1000 indicating antibody negativity. hyper-CVAD = hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone. HD-MTX-AraC = high dose methotrexate and cytarabine, pos. = positive, neg = negative [Colour figure can be viewed at wileyonlinelibrary.com]

Umbilical Cord Blood Bank allow the selection of HLA-compatible donors, but data related to CD36 expression are unavailable; and (3) only few reports are currently available on the transplantation of CD36-positive stem cells to CD36-negative patients.

The patient completed four cycles of hyper-CVAD followed by a two-year maintenance regimen consisting of ponatinib, vincristine, and PSL and is presently in molecular remission.

DISCUSSION

The CD36 antibody is known to cause PTR [6] and FNAIT and was recently found also to be involved in the pathogenesis of transfusion-related acute lung injury (TRALI) [7]. Six cases of type I CD36-deficiency with CD36 antibodies among Japanese blood donors who were all homozygous for the c.329_330delAC or c.949insA [7]. A Japanese blood donor whose plasma caused TRALI in the transfusion recipients had a high CD36 antibody titre and was also homozygous for c.329_330delAC [7]. These mutations are most frequently found in the Chinese population [8] while c.268C > T, the most common mutation in the Japanese population, is usually not associated with antibody production [7]. The present patient was found to be harbouring c.329_330delAC and c.949insA compound heterozygous mutations. She had a high CD36 antibody titre, which was thought to be dependent on antigenic exposure during her two pregnancies and CD36-positive PC transfusion.

The PTR in our case was caused by both CD36 and HLA antibodies, a fact which made it difficult to locate a compatible PC donor. However, this problem was solved through close collaboration with the JRCBC, allowing chemotherapy to be performed. Regarding the HSCT, there were concerns about transplantation-related complications, such as graft failure caused by CD36 expression on HSC and the requirement for frequent and long-term supplies of HLA-compatible, CD36-negative PC. There are only two case reports of CD36-positive HSCT in CD36-negative patients, one in Japan [9] and the other in China [10]. Successful engraftment was observed in both cases; in the latter case, the patient's platelets and monocytes were confirmed to be CD36-positive simultaneously with engraftment. On the other hand, the former case developed idiopathic pneumonia syndrome after engraftment (personal communication from Dr. Ito, Reference [9]); thus, the possibility of a TRALI-like syndrome due to an infusion of CD36-positive stem cells cannot be completely disregarded.

A previous report described two patients in whom TRALI developed after a plasma transfusion from the same donor [11]. TRALI may develop through monocyte activation in the alveolar tissue via binding with the CD36 antibody, which releases inflammatory mediators (LTB4 and TNF-a) and results in alveolar endothelial cell activation, in turn causing leakage [11]. Additionally, endothelial cells (EC) also express the CD36 antigen; thus, EC may possibly be damaged via binding with the CD36 antibody. Thus, if a CD36-positive HSCT is performed in a CD36-negative patient, the infused mononuclear cells trapped in the lungs might be activated by the CD36 antibody in the patient's plasma, leading to a TRALI-like syndrome through a similar mechanism.

If donor-specific anti-HLA antibody (DSA) is identified in the patient's plasma, pre-transplant antibody attenuation procedures, such as antibody elimination via plasmapheresis, inhibition of antibody production using rituximab or bortezomib, IVIG administration, and antibody neutralization by transfusions of PC expressing the corresponding HLA, are recommended. While there are at present no reports focusing on these methods, the current view is that desensitization should be performed in patients with DSA [12]. In the present patient, the CD36 antibody titre decreased after rituximab therapy, suggesting that this drug may be used with good results for pre-transplant desensitization to the CD36 antibody.

For HSCT indications in patients with type I CD36-deficiency with CD36 antibodies, confirming CD36 expression in potential donors is crucial. A CD36-negative patient in the US successfully received a transplant from a CD36-negative sibling [13]. Thus, ideally, CD36-negative donors should be selected for allogeneic (allo-) HSCT in CD36-negative patients with CD36 antibodies. Presently, neither the HSC donors registered to the JMDP nor those registered to the Umbilical Cord Blood Bank are being tested for CD36 expression, making it very difficult to locate CD36-negative allogeneic HSC donors. However, even if the patient does not have the CD36 antibody, a CD36-positive HSCT may be successful given appropriate management with transfusions of CD36-negative PC.

Based on these considerations, chemotherapy without HSCT was indicated in the present case. The patient is currently maintaining molecular remission after completing maintenance therapy. The MD Anderson Cancer Center reported relatively good results of the combination of hype-CVAD with ponatinib in Ph + ALL patients, with a three-year survival rate of 76% and no difference between the survival rates of patients with or without HSCT [14]. Thus, this chemotherapy regimen offers a good alternative regimen for patients in whom HSCT is contraindicated.

CONCLUSION

In the present patient with Ph + ALL with type I CD36-deficiency, an appropriate transfusion schedule and close collaboration with the JRCBC enabled safe chemotherapy administration. Considering the extremely high incidence of CD36 deficiency in Japan, implementing testing for CD36 expression on HSC is crucial to making CD36 and HLA-compatible donors more readily available.

ACKNOWLEDGEMENTS

We express our special gratitude to Dr Takuo Ito of the National Hospital Organization, Kure Medical Center and Chugoku Cancer Center for providing clinical information on the patient.

All authors contributed to interpretation of data and critically reviewed and approved the manuscript. M.M., K.K., M.T., K.I., K.Y., H. K. and Y.K. discussed the purpose and structure of the manuscript. K.S., K.T., M.A., and M.M. performed antibody testing and genetic analysis. M.M., N.W-O. and N.H.T. wrote the manuscript.

JO and GM contributed research design. GC and GDL conducted deta collection. FL and AL prepared and analysed data.

I and the other authors belonging to the Tokyo Metropolitan Tama medical center were the team that handles patient care. The authors of the Japanese Red Cross Kanto- Koshinetsu Blood Center were in charge of genetic and antibody testing on the patient and made efforts in supplying compatible platelets.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ORCID

Marina Matsui https://orcid.org/0000-0002-6514-9127
Naoko Watanabe-Okochi https://orcid.org/0000-0002-2362-257X
Nelson Hirokazu Tsuno https://orcid.org/0000-0002-7619-6500

REFERENCES

1. Bio GPS. Gene report. Situation reports October 2020. Available from http://biogps.org/#goto=genereport&id=948
2. de Wolf JT, Muller EW, Hendriks DH, Halie RM, Vellenga E. Mast cell growth factor modulates CD36 antigen expression on erythroid progenitors from human bone marrow and peripheral blood associated with ongoing differentiation. Blood. 1994;84:59–64.
3. Chen L, Gao Z, Zhu J, Rodgers GP. Identification of CD13+CD36+ cells as a common progenitor for erythroid and myeloid lineages in human bone marrow. Exp Hematol. 2007;35:1047–55.
4. Yanai H, Chiba H, Fujiwara H, et al. Phenotype-genotype correlation in CD36 deficiency types I and II. Thromb Haemost. 2000;84:436–41.
5. Tomiyama Y. Anti-platelet antibodies (Allo-, Iso-, or auto-antibodies) and their clinical relevance. Jpn J Transf Cell Ther. 2018;64:681–7.
6. Ikeda H, Mitani T, Ohnuma M, et al. A new platelet-specific antigen, Nak^{a}, involved in the refractoriness of HLA-matched platelet transfusion. Vox Sang. 1989;57:213–7.
7. Ando M, Nakajima F, Kamada H, et al. Defective CD36 mutations in anti-Nak^{a} antibody-positive subjects associated with transfusion-related acute lung injury (TRALI). Jpn J Transf Cell Ther. 2016;62:587–91.
8. Xu X, Liu Y, Hong X, et al. Variant of CD36 gene and their association with CD36 protein expression in platelets. Blood Transfus. 2014;12:557–64.
9. Ito T, Daitoku S, Nishi Y, et al. Anti-CD36 Allo-antibodies in a patient undergoing hematopoietic stem cell transplantation. Paper presented at: The 32th annual meeting of the Japan Society for Hematopoietic Cell Transplantation 2010; PS-1-50: 245.
10. Zhou Y, Li LL, Zhong ZL, et al. Anti-CD36 mediated platelet transfusion refractoriness and related cases after stem cell transplantation. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2018;26:541–6.
11. Nakajima F, Nishimura M, Hashimoto S, Okazaki H, Tadokoro K. Role of anti-Naka antibody, monocytes and platelets in the development of transfusion-related acute lung injury. Vox Sang. 2008;95:318–23.
12. Ciurea SO, Cao K, Fernandez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus guidelines for the detection and treatment of donor specific anti-HLA antibodies (DSA)in Haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. 2018;53:521–34.
13. Culler EE, Hillyer CD, Haight AE, Castillejo M, Josephson CD. CD36 immunization in a patient undergoing hematopoietic stem cell transplantation. Pediatr Blood Cancer. 2008;50:660–2.
14. Jabbour E, Short NJ, Ravandi F, et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: Long-term follow-up of a single-centre, phase 2 study. Lancet Haematol. 2018;5:618–27.

How to cite this article: Matsui M, Kajiwara K, Tsukada M, et al. A case of Philadelphia chromosome-positive acute lymphocytic leukaemia with type I CD36 deficiency. Vox Sang. 2021;1–5.

Received: 4 February 2021 | Revised: 12 April 2021 | Accepted: 14 April 2021

DOI: 10.1111/vox.13122

SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Naturally occurring anti-Kp^a in an infant with recurrent bacterial infection: A case report and review of the literature

Dhivya Kandasamy | **Shamee Shastry** | **Deepika Chenna** | **Ponniah Amutha Prethika** | **Ashwinkumar Vaidya** | **Vinu Rajendran**

Department of Immunohematology & Blood Transfusion, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India

Correspondence
Dhivya Kandasamy, Department of Immunohematology & Blood Transfusion, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India
Email: docdhivya99@gmail.com

Abstract

Naturally occurring anti-Kp^a antibody is extremely rare and was first reported in 1957, named after the first producer 'Penney'. However, the subsequent anti-Kp^a reports presented were all anti-Kp^a due to isoimmunization. Individuals with severe bacterial infections particularly Gram-negative bacteria are known to be capable of producing cross-reactive antibodies against Kell blood group system. However, such uncommon antibodies like anti-Kp^a can be easily missed in routine pre-transfusion testing unless the panel cells containing low incidence antigen are used for antibody screening. Here, we report a case of naturally occurring anti-Kp^a antibody, identified incidentally during pre-transfusion testing of a 12-month-old female infant with the diagnosis of Niemann–Pick disease and recurrent bacterial (*Escherichia coli*) infection.

KEYWORDS
Allo-anti-Kp^a, bacterial infection, cross-reactive antibodies, low incidence antigen

INTRODUCTION

The Kell system located on chromosome 7 is a complex and highly immunogenic blood group system. It comprises 35 antigens which are protein in nature and are well developed in early fetal life. Kp^a is a low incidence antigen, a component of a triplet of antithetical (Kp^a, Kp^b, Kp^c) antigens of the Kell blood group system. The frequency of Kp^a antigen accounts for less than 2% of individuals globally and varies from 0 to 0·01% in Asian ancestry [1,2]. Though the original report of anti-Kp^a was naturally occurring [3], the later anti-Kp^a reports were identified in patients who had *a prior* red cell sensitizing event [4–15]. Many Gram-negative intestinal flora is known to act as primary immune stimuli for the development of naturally occurring antibodies, and several authors have shown *Escherichia coli* infection to stimulate antibody production against K antigen of Kell blood group system [16–18]. Although anti-Kp^a is an antibody of less clinical importance, it can cause haemolytic transfusion reaction (HTR) and haemolytic disease of the fetus and newborn (HDFN) [12,15]. Here, we report a case of naturally occurring anti-Kp^a antibody, identified in a female infant diagnosed to have lysosomal storage disorder with a significant past medical history of recurrent systemic infections caused by *E. coli*. This report on naturally occurring anti-Kp^a is first of its kind from India and highlights the fact that environmental factors like microbial exposure during early years of life can stimulate the production of non-ABO antibodies like anti-Kp^a.

CASE REPORT

A female infant of 12 months old with features of severe anaemia and failure to thrive was referred to our tertiary care centre for further evaluation and management. The child was healthy until 2 months of age and later developed frequent episodes of lower respiratory tract infection (LRTI) associated with abdominal distension, jaundice and loose stools, positive for *E. coli* during fourth and sixth months of life. On further extensive investigation, the child was diagnosed to have Niemann–Pick disease, a genetic lysosomal storage disorder. Due to low haemoglobin (Hb) level of 6 g/dl, we received a transfusion request for one unit of packed red blood cells (PRBC). The child had no previous history of blood transfusion or fetal anaemia following

TABLE 1 The review of the literature on case reports of anti-Kp^a from 1957 through 2020

	Anti-Kp^a—Isoimmunisation		
Anti-Kp^a - Naturally occurring	**Hemolytic disease of the newborn**	**Transfusion reaction**	**Pretransfusion testing***
Allen Jr and Lewis [3]	Jensen [4]	Koshy et al. [12]	Mangwana and Gangwar [2]
	Geczy et al. [5]	Padmore et al. [13]	Pahuja et al. [9]
	Costamagna et al. [6]	Sunassee et al. [14]	Jain et al. [10]
	Rossi et al. [15]		Makroo et al. [11]
	Tuson [7]		
	Brumbaugh et al. [8]		

*Indian reports of anti-Kp^a in Thalassemia [9, 10], Non-Hodgkin's lymphoma [2] and general patients [11].

childbirth. The historical blood group and the pre-transfusion testing revealed the patient's blood group as B Rh D positive with a discrepancy in serum grouping wherein an additional 1+ strength reaction was observed against the commercial O and B pooled cells. Blood grouping was performed by column agglutination technique (CAT) in an automated platform (AutoVue, Ortho Clinical Diagnostics). The grouping discrepancy got resolved with conventional tube technique (CTT), after incubation of the sample at 37°C, suggestive of cold reactive antibody. However, antibody screening of patient's sample using 3 cell panel (Bio-Rad Diamed GmBH, Cressier FR, Freiburg, Switzerland) in LISS/Coomb's IgG + C3d gel card showed 2+ reaction with the possible specificity for E, Jk^b, Kp^a or Le^a antibody, while autocontrol (AC) and direct antiglobulin test (DAT) demonstrated a negative reaction. The subsequent antibody identification using 11 cell panel (Bio-Rad Diamed GmBH, Switzerland) confirmed the antibody specificity as Kp^a antibody. Upon minor phenotyping of patient's red cells for Kp^a antigen, it was found negative. As Kp^a is usually IgG, the antibody screening of mother's serum was performed to rule out if a passive transfer of antibody from mother to child had occurred but was found negative. The above findings confirmed the presence of naturally occurring low incidence Kp^a alloantibody, likely to be stimulated by the recurrent systemic infection present since the early years of life.

The patient was transfused with one unit of group-specific, cross-match compatible PRBC unit which was negative for Kp^a antigen. The patient was tagged as positive for Kp^a antibody in the department software and the same was communicated to the patient's parents through an antibody card specifying the presence of anti-Kp^a to aid future transfusion support. However, the IgM or IgG characterization of the antibody could not be performed due to inadequacy of the sample volume received.

Concurrently, we reviewed the literature on anti-Kp^a in PubMed, Scopus and Embase databases using keywords like anti-Kp^a, all o anti-Kp^a and naturally occurring anti-Kp^a in the last 63 years (1957 through 2020) to gain insight on this rare antibody (Table 1).

DISCUSSION

Anti-Kp^a is an uncommon antibody, and it is considered as clinically less significant due to the low incidence of Kp^a antigen which varies from 0·01 to 2% globally [2]. In general, antibodies against the Kell blood group system are highly immunogenic and have the potential to stimulate alloantibody formation in patients who lack the corresponding antigen [1]. However, the original case report of anti-Kp^a by Allen and Lewis, in 1957, named after the first producer 'Penny' was naturally occurring and this is reaffirmed by the present report.

Although there is no evidence for Kell system to act as a receptor for microbial pathogens, there are several reports in the literature where severe bacterial infections by organisms like *Escherichia coli 0125815*, *Morganella morganii*, *Enterococcus faecalis*, *Streptococcus faecium* and *Mycobacterium tuberculosis* are shown to be capable of stimulating cross-reactive antibodies against K antigen of Kell blood group system in following ways: (1) release of Kell-like soluble substances by the organism, (2) the metabolites of an organism having Kell-like activity and (3) the organism can carry K1-like antigen. However, the exact location of the antigen and their biochemical relationship to Kell-like antigen are not yet available [16–18]. These findings suggest that Gram-negative bacteria can also stimulate antibody against other antigens of the Kell blood group system. Hence, the significant history of recurrent infection by *E. coli* in our case favours the possibility of naturally occurring antibody to have developed following microbial exposure.

The red cell alloimmunization can occur through blood transfusion, transplantation or pregnancy. However, the early onset of transfusion before 3 years of age is associated with a lower frequency of alloantibody formation especially among chronically transfused patients. Further, the earliest sensitization in an individual can occur after 10 transfusions [19,20]. Accordingly, the earlier anti-Kp^a case reports from India were noted in either multiply transfused patients like thalassaemia, non-Hodgkin lymphoma or following HTR or HDFN, as shown in Table 1. However, the present case, with no red cell immunization history but with a significant bacterial infection present since early years of life, highlights the association of microbial flora in the development of naturally occurring non-ABO blood group antibody. Further taking into account that antibodies to the KEL blood group system antigens are usually IgG and human maternal antibodies take about 6 to 12 months period to wane off [21], there is a possibility for passive transfer of anti-Kp^a antibodies from mother to child. However, this hypothesis got eliminated in our case as both maternal antibody screening and Kp^a minor phenotyping of the infant were negative. Unfortunately, the complete family pedigree analysis on the inheritance of Kp^a antigen could not be performed to substantiate our hypothesis. Understanding the nature of alloantibody as in naturally occurring or developed after red cell alloimmunization is important to

identify the potential immune responder, capable of developing multiple alloantibodies with further phenotype unmatched transfusions.

Concerning the role of immunohematological testing in detection of red cell alloantibody, Padmore et al. have reported on the implication of anti-Kp[a] in causing an acute extravascular haemolytic reaction in a patient, where the antibody was missed by electronic cross-match [13]. Therefore, despite the nature of antibody, red cell antibody if detected in pre-transfusion testing and found to be reacting at 37° Celsius in antihuman globulin (AHG) phase, including those antibodies against uncommon antigen, should undergo extensive immunohematological work-up to ensure safe blood transfusion.

The present report adds further evidence to the occurrence of anti-Kp[a] as a naturally occurring antibody especially in patients with the history of recurrent bacterial infections. Although it is a rare antibody, utmost importance should be given in identification of such uncommon antibodies in pre-transfusion testing of alloimmunized and those patients with significant microbial exposure. The prompt documentation and communication of the unexpected red cell antibody identified to the patient help in the prevention of immune-related complication as well as delay in future transfusion.

CONFLICT OF INTEREST

None declared.

ETHICAL ISSUES

Informed consent of the patient was obtained to access clinical and laboratory details.

ORCID

Dhivya Kandasamy https://orcid.org/0000-0002-3175-4652
Shamee Shastry https://orcid.org/0000-0001-9796-2326
Deepika Chenna https://orcid.org/0000-0003-1461-5498
Ashwinkumar Vaidya https://orcid.org/0000-0002-2711-6965
Vinu Rajendran https://orcid.org/0000-0002-8703-6473

REFERENCES

1. Storry JR. Other blood group systems and antigens. In Fung MK, Grossman BJ, Hillyer CD, Westhoff CM eds. Technical Manual. 18th ed. Bethesda, MD, AABB; 2014:337–66.
2. Mangwana S, Gangwar V. Anti Kp[a] alloantibody: development of a rare alloantibody in a non-Hodgkin's lymphoma patient of Indian origin. Asian J Transfus Sci. 2018;12:81–4.
3. Allen FH Jr, Lewis SH. Kpa (Penney), a new antigen in the Kell blood group system. Vox Sang. 1957;2:81–7.
4. Jensen KG. Haemolytic disease of the newborn caused by anti-Kp[a]. Vox Sang. 1962;7:476–8.
5. Geczy A, Ennis J, Golian PR. A further example of Anti-Kpa. Transfusion. 1965;5:89–91.
6. Costamagna L, Barbarini M, Viarengo GL, Pagani A, Isernia D, Salvaneschi L. A case of hemolytic disease of the newborn due to anti-Kpa. Immunohematology. 1997;13:61–2.
7. Tuson M, Hue-Roye K, Koval K, Imlay S, Desai R, Garg G, et al. Possible suppression of fetal erythropoiesis by the Kell blood group antibody anti-Kp(a). Immunohematology. 2011;27:58–60.
8. Brumbaugh JE, Morgan S, Beck JC, Zantek N, Kearney S, Bendel CM, et al. Blueberry muffin rash, hyperbilirubinemia, and hypoglycemia: a case of hemolytic disease of the fetus and newborn due to anti-Kp (a). J Perinatol. 2011;31:373–6.
9. Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M, Vasudeva S. Anti Kp[a] antibody: a rare alloantibody in a multitransfused thalassemic of Indian origin. Indian J Pathol Microbiol. 2010;53:575–6.
10. Jain R, Choudhury N, Chudgar U, Harimoorthy V, Desai P, Perkins J, et al. Detection and identification of red cell alloantibodies in multiply transfused thalassemia major patients: a prospective study. Indian J Hematol Blood Transfus. 2014;30:291–6.
11. Makroo RN, Bhatia A, Hegde V, Chowdry M, Thakur UK, Rosamma NL. Antibody screening & identification in the general patient population at a tertiary care hospital in New Delhi, India. Indian J Med Res. 2014;140:401–5.
12. Koshy R, Patel B, Harrison JS. Anti-Kpa-induced severe delayed hemolytic transfusion reaction. Immunohematology. 2009;25:44–7.
13. Padmore R, Berardi P, Erickson K, Desjardins D, Giulivi A, Tokessy M, et al. Acute extravascular hemolytic transfusion reaction due to anti-Kpa antibody missed by electronic crossmatch. Transfus Apher Sci. 2014;51:168–71.
14. Sunassee A, Mackey J, Anderson KA, Huntington MK. Acute hemolytic transfusion reaction due to anti-Kp[a] antibody. S D Med. 2018;71:222–3.
15. Rossi KQ, Scrape S, Lang C, O'Shaughnessy R. Severe hemolytic disease of the fetus due to anti-Kpa antibody. Int J Blood Transfus Immunohematol. 2013;3:19–22.
16. Cooling L. Blood groups in infection and host susceptibility. Clin Microbiol Rev. 2015;28:801–70.
17. Marsh WL, Nichols ME, Oyen R, Thayer RS, Deere Wl, Freed PJ, et al. Naturally occurring anti-Kell stimulated by *E. coli* enterocolitis in a 20-day-old child. Transfusion. 1978;18:149–54.
18. Savalonis JM, Kalish RI, Cummings EA, Ryan RW, Aloisi R. Kell blood group activity of gram-negative bacteria. Transfusion. 1988;28:229–32.
19. Hendrickson JE, Tormey CA. Understanding red blood cell alloimmunization triggers. Hematology Am Soc Hematol Educ Program 2016;1:446–51.
20. Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassemia. Vox Sang. 1990;58:50–5.
21. Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Front Immunol. 2014;5:446.

How to cite this article: Kandasamy D, Shastry S, Chenna D, Prethika PA, Vaidya A, Rajendran V. Naturally occurring anti-Kp[a] in an infant with recurrent bacterial infection: A case report and review of the literature. Vox Sang. 2021;1–3.

Received: 26 April 2021 | Accepted: 27 April 2021

DOI: 10.1111/vox.13129

INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on Policies and Practice for Transfusion of ABO and RhD Non-Identical Platelets: Summary

Rebecca Cardigan | Helen V. New | Lise Estcourt | Eugene Zhiburt | Rounak Dubey | Jesper Bengtsson | Magnus Jöud | Carlos Castillo | Joan Cid | Miquel Lozano | Dhana Gounder | Peter Flanagan | Sarah Morley | Gwen Clarke | Dana Devine | Salwa Hindawi | Aqeel AlOtaibi | Carolina Bonnet Bub | Jose Mauro Kutner | Toshiyuki Ikeda | Naoko Goto | Hitoshi Okazaki | Magali J. Fontaine | Jeremiah Pasion | Linda Song | Tom Latham | Jean-Louis Kerkhoffs | Masja de Haas | Jaap Jan Zwaginga | Birgit S. Gathof | Katharina Ommer | France Pirenne | Michel Raba | Anne Francois | James Daly | Tanya Powley | Nancy Dunbar

Transfusion of ABO major incompatible platelets (where the recipient has antibodies against the ABO group of the transfused platelets) has been shown to be associated with lower platelet count increments following transfusion [1], although this does not appear to translate to a difference in bleeding outcomes [2]. Transfusion of minor ABO incompatible platelets (where the suspending plasma of the platelet concentrate is not compatible with the ABO group of the recipient's red cells) has been associated with increased risk of haemolytic transfusion reactions. This risk is likely to be lower for platelets stored in platelet additive solution due to the reduced volume of plasma.

Whilst the preference, therefore, is that patients are transfused ABO identical platelets, this is not always possible in emergency situations. Moreover, transfusion of ABO incompatible platelets may also be required to ensure, across the supply chain, that platelets are available when needed without significant wastage of a product with short shelf-life (5–7 days in most countries). For this reason, transfusion of ABO incompatible platelets is not an infrequent occurrence, and thus, many centres have policies in place to mitigate risk to recipients. A previous international survey published in 2010 reported considerable variation in international practice in relation to the transfusion of ABO incompatible platelets [3]. Since that time, platelet production practice has evolved, especially with increased use of platelet additive solution either alone or in combination with pathogen inactivation.

In addition to ABO group, consideration must be given to compatibility of platelets for RhD. Platelets themselves do not express RhD, but are contaminated with a small amount of red cells. The level of red cell contamination is influenced by the method of production, with apheresis platelets reported to contain lower levels than those produced by whole blood [4]. Transfusion of RhD-positive platelets to RhD-negative recipients can result in alloimmunisation, the likelihood of which is related to the type of platelet preparation and factors in the recipient such as immunosuppression [5]. Therefore, many centres have policies to define which recipients should receive RhD-negative platelets. The principal concern is mitigating the risk of alloimmunisation in women of child-bearing potential, potentially leading to haemolytic disease of the foetus and newborn. Where RhD-negative platelets are not available for patients for whom they are indicated, centres may have policies for prophylactic administration of anti-D following transfusion of RhD-positive platelets.

The aim of this International Forum was to assess current international policies for the transfusion of ABO or RhD non-identical, "out of group," platelets in relation to how platelets are produced and tested.

We received responses from 15 centres, with a wide geographical spread. Many respondents were reporting national guidance.

TYPE OF PLATELET COMPONENTS TRANSFUSED

Question 1 Please describe the type of platelets products you supply.

Data are summarized in Table 1.

In terms of platelet production methods, there was a mix of apheresis and whole blood-derived platelets reported, with a trend towards a higher proportion of platelets produced from whole blood in European Countries as well as New Zealand, Australia, India and Brazil. Most centres (11/15) stored all or part of their platelet supply in PAS or were planning on doing so in the near future. Since the last published survey in 2010 by the BEST group [3], there appears to have been an increase in the use of PAS either alone, or in conjunction with pathogen inactivation. Of the 13 respondents that produce whole blood derived platelets, the majority [6] do so by manual or semi-automated methods (e.g. presses), with 3 using Reveos and 1 (France) using Terumo Automated Centrifuge and Separator (TACSI). Most had similar specifications for platelets for neonatal/infant use as those for adults (Table 1), but in 8/15, these were stated to be provided at smaller volume and/or concentrated. Two responders stated they provide CMV seronegative platelets for this patient group (Spain and UK), and UK guidelines require that this product is free from clinically significant irregular blood group antibodies including high titre anti-A and B. Some countries provide additional mitigations for ABO non-identical transfusions for neonates or children (Table 2).

ABO

Question 2 Do you have national/local clinical polices with respect to transfusion of ABO non-identical platelets? What percentage of platelet transfusions are ABO non-identical? If ABO identical platelets are not available, is priority given to compatibility of ABO group of platelet (avoiding major incompatibility) or suspending plasma with recipients red cells (avoiding minor incompatibility)?

TABLE 1 Type of platelets produced

	Type of platelets used		Whole blood platelets		Storage media used		
Country	Apheresis	Whole blood	Method of processing	No. of donations	Apheresis	Whole blood	Different specification for neonatal/infant platelets?
Australia	36%	64%	Semi-automated PRP or BC.	4	60%–70% PAS-E.		Apheresis, smaller volume.
Brazil	95%	5%	Manual, PRP.	6	Plasma.		No
Canada	25%	75%	Semi-automated, BC.	4	Plasma.	Plasma (moving to PAS).	No
England	50%	50%	Manual, BC.	4	Plasma.	65% PAS-E.	Apheresis, smaller volume. CMV neg, micro neg in past two years. Low titre for anti-A/B, negative for clinically significant irregular blood group antibodies.
France	30% PI	70%	Automated (TACSI) and PI.	5–8	70% PAS-C.		Apheresis, smaller volume. Select the most concentrated packs.
Germany	70%	30%	Manual BC.	5, moving to 4.	Plasma (moving to PAS).	PAS-C.	No
India	10%	90% PRP	Manual, PRP.	1	Plasma.		No
Japan	100%	—	—	—	Plasma.	—	Smaller volume (provided also for small children.
Netherlands	10%	90%	Semi-automated BC.	5	65% PAS-E.		Smaller volume.
New Zealand	30%	70%	Manual, BC.	4	60%–70% PAS-E.		Apheresis PC stored in plasma (plan to move to PAS).
Russia	70%	30%	Manual, BC.	4–5	Plasma or PAS-E.		No, but are usually irradiated.
Saudi Arabia	10%–20%	80%–90%	Automated (Reveos).	4–6	Plasma.		Smaller volume, ABO compatible.
Spain	5%	95%	Automated (Reveos).	4–5	Plasma.	65% PAS-C.	CMV neg donors, reduced size pools (from 3 donors).
Sweden	7%	93%	Automated (Reveos).	4 (3–6)	60%–65% PAS-E.		No, split units from the same donation used for one patient.
USA	100%	—	80% PI in PAS, 20% non-PI.	—	10% in plasma, 90% in PAS.	—	No

Abbreviations: BC, Buffy coat; PAS, Platelet additive solution; PI, pathogen inactivation; PRP, Platelet rich plasma.

Question 3 What strategies are taken to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility and are these dependent upon the type of platelet product?

Data are summarized in Table 2.

Most centres (9/15) used national guidelines or policies that describe requirements for ABO matching of platelets and the clinical use of non-identical platelets. Other centres either had local policies or no agreed policy in place. The majority of responses indicated that the preference was to give ABO identical platelets if available, but it

TABLE 2 Policy and practice for platelet ABO matching

Country	National or local policies?	% ABO non-identical transfusions	Priority given to minor or major compatibility	Risk mitigation for minor mismatch	Frequency of HTR due to ABO incompatible transfusion
Australia	National	Not known	Minor	Storage in PAS. Low-titre for anti-A/B.	No cases since the introduction of anti-A/B testing of donations.
Brazil	National	36%	NR	Low-titre for anti-A/B.	None seen in years but are reportable to HV scheme.
Canada	National	20%	Minor	Low-titre for anti-A/B. Volume reduction.	2 reports since 2017.
England	National	10%–15% likely higher for neonates.	Minor	Low titre for anti-A/B. Pooled platelets in PAS. Avoid group O for non-O patients.	1:625000. None since 2016 - PAS introduced for pooled platelets. No fatalities since testing standardized in 2008.
France	No policy	16% major.	Major	Low-titre for anti-A/B.	Reportable but very rare over past 8 years.
Germany	National	—	Major	Storage in PAS.	13 fatal cases 2000–2017 nationally, 1:600000 platelets.
India	Local	5%	Minor. Avoid group O for latter.	Apheresis platelets, volume reduced if there is time.	None observed at centre last year, but reportable to national HV.
Japan	National	0% apart from HLA matched - 30%.	For HLA matched, minor.	Low-titre for anti-A/B. Washing for HT units or for children. Hospitals can volume reduce.	1:2million PC overall, 1:19108 for HLA matched platelets.
Netherlands	National	5%	Major. Group O RhD neg used in trauma packs	All PC in PAS. Low-titre for anti-A/B anti-A/B for neonates, infrequently volume reduction.	None in 2018.
New Zealand	National	23%	Major	Storage in PAS.	6 reports between 2005–2012 all due to group O PC. None since 2012, PAS introduced 2010–11.
Russia	National	21%	Not stated	Storage in PAS.	NR
Saudi Arabia	Local	20%	Not stated	Volume reduction to 30% for some patients - especially for neonates.	None reported but there is a system to report to national body.
Spain	None	NK	Major - risk of minor reduced by suspension in PAS	Storage in PAS. No testing for anti-A/B.	Not observed in past 30 years, reportable to national HV scheme.
Sweden	Local	22%	Minor but sometimes major.	Storage in PAS for BC PC, apheresis donors tested for anti-A/B. May also wash apheresis PC if from an HLA matched donor with HT antibodies.	Not observed in centre in last 15 years. One case reported to national HV scheme in past 10 years.
USA	Local	42%	Minor	Storage in PAS –OR-volume reduced to 50 ml.	Observed, so considering testing for anti-A/B.

was noted that this was not always possible, and this may be particularly the case for specific groups of patients such as neonates. Some blood providers preferentially collect platelets from group O and A donors to aid inventory management and supply, and in this case, group AB and B recipients will be more likely to receive ABO non-identical platelets. If ABO identical platelets are not available, then the preference for prioritizing compatibility of the platelets or plasma that they are suspended in is mixed. Six centres give priority to ensuring the compatibility of plasma with recipients red cells (minor), whereas five preferentially consider the ABO compatibility of the platelets themselves (major). In two centres, it depended on the platelet component. The majority prioritizing minor compatibility indicated that their rationale was that the risk of a haemolytic transfusion reaction was reduced by platelets being suspended in PAS and/or testing to remove high titre units. The proportion of ABO non-identical transfusions (as defined by centres) is typically about 20%. However, ABO incompatible transfusions are a rare event in Japan unless platelets are HLA matched, whereas in the USA, it appears to be more common at over 40%. This is consistent with a recent study in 12 hospitals in the USA demonstrating that 46% of platelet transfusions are ABO non-identical—16% minor incompatible [7]. Some respondents noted that the likelihood of ABO non-identical transfusion was increased for HLA matched platelets, where HLA matching may take preference over ABO compatibility.

In addition to many centres using PAS for all or part of their supply, 6/15 may volume reduce to limit the amount of plasma transfused, and 2 may wash HLA-matched platelets if ABO non-identical. 8/15 have implemented partial or universal testing of anti-A/B for donations destined for platelet production in specific situations including for ABO non-identical transfusions, or for neonates. The method and cut offs used for testing are summarized in Table 3. All the sites that test do so on samples from the donor, apart from the Netherlands who test the final product for neonatal transfusion. There are data from small studies suggesting that the titre of anti-A/B for a given donor are stable over time [8], suggesting that one off testing of donors may be sufficient, but this has not been confirmed in large data sets and many operators (6/7 who responded) still test every donation. Most sites that test do so for IgM only (5/7 where stated), 2 also test IgG. The cut off used to determine whether a donation is "high titre" varies between 1/64 and 1/128 for IgM. It is well known that the methodology for testing affects the titre obtained, and therefore it is difficult to compare across sites [9].

The main reported effect of transfusion of ABO major incompatible platelets is a reduction in platelet count increment following transfusion, which appears to be a cumulative effect [1]. This difference is small and does not appear to relate to risk of bleeding [2]. Effects of ABO mismatch on other measures of platelet outcome such as survival are less clear [6]. Some studies suggest that major ABO incompatibility may result in a higher risk of developing HLA or platelet specific antibodies [10] or transfusion reactions [11]. In addition to the development of alloantibodies in the recipient to antigens they lack, the development of immune complexes due to anti-A and/or B present in the recipient with the cognate antigen on donor platelets or soluble antigen has been postulated as a possible mechanism [12, 13].

TABLE 3 Methods and cut off used for anti-a/B high-titre testing

Australia	All donations for platelets and plasma.	Donor	IgM at 1/32 and 1/64 (1:32 shown to be equivalent to 1/128 manual tube method). Apheresis platelet donors positive at 1/64 titred at 1/8000 and if positive deferred from platelet donation.	Yes	No. Labelled as low titre if they test as such.
Brazil	Platelet donors.	Donor	Gel card IgM for anti-A or B, 1/64 used as cut off.	Yes	No. Given on inventory report.
England	All donations.	Donor	Microtitre plate IgM anti-A and B cut off is 1/32 shown as equivalent to 1/128 manual saline tube method.	Yes	No. Labelled as low titre if they test as such.
France	All donations.	Donor	Microtitre saline IgM only >64 cut off.	Yes	No. Labelled with group compatibility.
Japan	Donations for HLA matched platelets if ABO non-identical.	Donor	1/128	Yes	No
Netherlands	Platelets for neonatal use.	Product	Saline tube for IgM, cut off 1/64, must be negative at 1/128.	Yes	No
New Zealand	All donations.	Donor	Micro-titre IgM and IgG using A1B cells. A > 50% reduction of the cell button defined as positive.	Yes	NR
Sweden	Apheresis donors.	Donor	Gel cards. IgM titres ≥64 and IgG titres ≥256 are considered high.	No - once	No. Labelled as high titre or low titre according to cut offs.

In the PLADO study, a multi-centre RCT assessing different prophylactic platelet dose regimens, the degree of ABO matching (major or minor) was not associated with any overall difference in platelet alloimmunisation [14] or transfusion related adverse events [15] such as fever, allergic reactions, and tachycardia. There was a trend towards lower risk of febrile reactions in ABO minor mismatched platelets, which the authors attributed to chance. In critically ill children, transfusion of ABO incompatible platelets is not associated with difference in count increments or transfusion reactions [16].

Question 4 Despite steps taken in Question 3, do you observe cases of haemolytic transfusion reactions (HTR) to platelets, are these reported in national hemovigilance schemes and so forth, how frequent are they?

Fatalities associated with a HTR associated with ABO non-identical platelet transfusion have been reported here and in the literature. In this survey, Germany report 13 fatal cases between 2000 and 2017. In the United States, seven fatal cases have been reported to the FDA between 2007 and 2018 [17]. The responses in this survey suggest that HTR associated with ABO non-identical platelet transfusion are rare events due to the mitigating actions in place although previously many of the centres/countries had reports. The residual risk of a HTR due to ABO non-identical platelet transfusion is estimated as 1:600,000 in Germany, 1:6250,000 in England, and 1:2 million in Japan. Several authors note, as might be expected, that this is more frequent with group O platelets to non-O recipients, due to higher titres of anti-A/B in group O donors.

The relationship between titre level and risk of haemolytic transfusion reactions is not absolute [18]; there are case reports of HTR with platelets that have tested as "low titre" especially in neonatal patients [19]. Additionally, the risk of platelets in additive solution is not zero, in part because for apheresis platelets about 100 ml of plasma from one donor remains in the platelet, and occasionally donors may have very high titres of anti-A/B. In addition to genetic factors that likely influence a donor's level of anti-A/B, environmental factors such as pneumococcal vaccination and pro-biotics have been reported to boost levels in some donors [20–22]. Recent data on a large range of other vaccines suggest that in the main, these have minimal effect [23]. Interestingly to mitigate the risk of very high levels, Australia re-test all donors who test "high titre" using their screening method at a very high titre of 1/8000; any donors testing positive at this level are deferred from platelet donation. Further, pooling and use of PAS would be expected to reduce the risk of HTR to lower than that for apheresis platelets as only a small volume of plasma from each donor will end up in the final product transfused—as an example, this is around 20 ml of plasma from each donor in buffy coat platelets in our country (UK). Comprehensive risk modelling using large data sets for PAS platelets and risk of HTR are lacking.

Nonetheless, the responding countries who have implemented routine screening of platelet donors for anti-A/B report that the risk of HTR is now very low based on data from their hemovigilance scheme; this includes large national providers such as the UK, France and Australia. The UK have not observed any fatal cases since the UK-wide standardization of routine testing of anti-A/B, as is also the case for Australia. New Zealand also note that they have not observed any cases of HTR associated with ABO non-identical platelet transfusion since the universal implementation of PAS.

RhD

Question 5 Do you have national/regional clinical polices with respect to transfusion of RhD-negative platelets? For which group(s) of patients are RhD-negative platelets indicated?

Nearly all those responding (12/13) prioritized the use of RhD-negative platelets for RhD-negative women of child-bearing potential, the definition of which, where given, varied between 45 and 55 years of age (Table 4). Several of these national policies also included RhD-negative children in this category. Policies for other RhD-negative patients varied. Japan does not have a national policy due to the low (<0.5%) frequency of RhD-negative individuals in the population. Russia does not take RhD into consideration when transfusing platelets.

Question 6 If RhD-negative platelets are not available for the above patients what mitigation steps are taken to reduce the risk of alloimmunisation if RhD-positive platelets are transfused?

In 10/15 if RhD-positive platelets are transfused to a RhD-negative females of child-bearing potential (as defined by their policy), then anti-D prophylaxis is routinely given/recommended. In a further 3/15, anti-D may be given at the discretion of the treating physician. Only New Zealand routinely use anti-D for male children in this situation. Interestingly, both New Zealand and France take into account the clinical status of the patient in the decision to administer prophylactic anti-D: this may not necessarily be given if patients are immunosuppressed. In addition to the volume of red cells transfused, recipient factors are thought to contribute to the likelihood of alloimmunisation to RhD. In healthy volunteers, even small doses of RhD-positive red cells are near certain (80%) to induce alloantibody formation in RhD-negative individuals [24]. However, the reported frequency of alloimmunisation when RhD-positive platelets are transfused to RhD-negative patients is reported to be relatively low at approximately 1.5% [25]. The latter study was a large multi-centre study that included all indications for platelet transfusion, as such a significant proportion of recipients were immunosuppressed either through treatment or pathology.

Question 7 Do you routinely assess the level of red cell contamination of platelets?

- By what method and what is the upper limit considered acceptable to issue platelets?
- Is this information on the label or shared with hospitals that use the platelets?

TABLE 4 Policy and practice for RhD matching

Country	National/ local policies	Patients for whom RhD neg indicated & policy, if not available	Assessment of red cell contamination (cut off applied)	Is alloimmunisation to RhD reported?
Australia	National	RhD-neg females of CBP. Anti-D unless immunosuppressed.	Visual–colour chart (1 ml red cells).	No. HV system captures alloimmunisation post-transfusion.
Brazil	National	RhD-neg females <45y. Anti-D.	Visual	Yes. Reportable to HV scheme.
Canada	Local	RhD-neg females <45y. May be extended to all children in some sites. Anti-D for women <45y, boys at physician discretion.	Visual – colour chart.	Yes. Reporting is voluntary.
England	National	RhD-neg females of CBP. RhD-negative boys <18, pre-existing anti-D antibodies, and transfusion-dependant adults. Anti-D for women of CBP.	Visual – colour chart ~4 x 10^9 RBC/L.	No. Anti-D in women of CBP reportable to HV scheme. No cases in past 10 years.
France	Local	RhD-neg females <50y. Anti-D unless immunosuppressed.	Visual	Yes. Recommended to test 1–4 months after RhD incompatible transfusion.
Germany	National	RhD-neg women of CBP. Children <14y. Anti-D.	FBC and QC by flow cytometry (3 × 10^9 RBC/L).	No
India	Local	RhD-neg women of CBP if available. Anti-D at discretion of treating physician	Visual (0.5 ml or 5 × 10^9).	No
Japan	None	RhD-neg patients, may use anti-D in rare cases of RhD pos to RhD-neg women of CBP, but use is off label.	Annual QC 10 per year.	None reported in last decade.
Netherlands	National	RhD-neg females <45. Anti-D except for <3 months old.	Visual (6 × 10^9 RBC/L).	Yes to national HV scheme.
New Zealand	National	RhD-neg females <55. Other RhD-neg patients if available. Anti-D for females <55 and male children. Underlying condition/treatment considered in risk of alloimmunisation.	No routine testing but data from studies.	No. 2y analysis where RhD pos given to RhD-neg patients without anti-D showed 1.4% alloimmunisation.
Russia	None	D, C, c, E, e, and K are not taken into account when transfusing platelet concentrates obtained by apheresis or using an additive solution or pathogen-reduced platelet.		No
Saudi Arabia	Local	All RhD-neg patients but especially females of CBP. Anti-D.	Visual colour chart (>2 ml red cells)	Reportable to local HV scheme, not all hospitals will have this.
Spain	Local	RhD-neg women of CBP, anti-D for females<50.	Visual + QC of PC by flow cytometry	No, but as most recipients also on chronic red cell support should be picked up in pre-transfusion testing.
Sweden	Local	All RhD-neg patients. For women of CBP and children RhD pos must be approved by a physician. For trauma/major bleeding, RhD pos may be used regardless of group/ age/sex of recipient. Anti-D for women of CBP and children.	Visual	No Risk considered low
USA	None	Physician contacted to consider anti-D	Visual	No

Virtually all responding participants (12/15) perform a visual inspection of platelets and have a colour chart to prevent those grossly contaminated with red cells being issued, but the cut offs used vary. The American Association of Blood Bank guidelines are followed by two respondents, which state that if platelets appear to contain ≥2 ml of residual red cells, making the component appear pink to salmon in colour, compatibility testing with the recipient's plasma is required [26]. India and Australia use a cut off of 0.5 ml and 1 ml, respectively, whereas Germany, England, and the Netherlands use cut offs that equate to 3, 4, and 6 × 10^9 RBC/L, respectively. Spain and

Germany are the only respondents that routinely accurately measure the red cell contamination of platelets by flow cytometry.

Curiously, there are no European guidelines for routine quality control of red cell contamination of platelets, whereas guidelines state that prior to freezing, fresh frozen plasma should contain $<6 \times 10^9$ RBC/L [27]. This is somewhat surprising as the risk of alloimmunisation caused by RhD incompatible plasma is lower than that of platelets. First, levels of red cells in plasma prior to freezing are lower than in platelet concentrates, and second, red cell stroma following thawing is considered likely less immunogenic than intact red cells [24]. Consequently, in some jurisdictions, frozen–thawed plasma is transfused irrespective of RhD group.

For platelets, it is unclear what minimum number of red cells is likely to result in the formation of anti-D when RhD-positive platelets are transfused to RhD-negative recipients. Small volumes of pure red cells of 0.5 ml or above are known to result in 80% of healthy volunteers developing anti-D, 20% being non-responders [28]. Before the advent of current antibody production techniques, this was used as a method of harvesting anti-D for purification and therapeutic use. The minimum quantity of red cells required to result in anti-D formation following transfusion is frequently quoted as being 30 μl of pure red cells based on a study from 1970 [29]. However, in this latter study, volunteers were actually given 10 μl of red cells on three occasions, 2 weeks apart and thus the number of RBC that may cause primary sensitisation may be lower than cited. Thirty microliters of pure red cells is equivalent to approximately 0.8×10^9 RBC/L in the volume of a unit of platelets and much lower than most centres' cut off for the acceptable levels of RBC in a unit of platelets described here.

Additionally, as well as intact red cells, platelets contain red cell microparticles that retain RhD antigens, the number of which varies by product type [30, 31]. However, red cell microparticles are not usually assessed by most methods that measure intact red cells. Intact red cells are known to be generally present at lower levels in apheresis compared to whole blood derived platelets [4, 32]. Contaminating red cell levels in buffy-coat derived platelets have been demonstrated to be substantially lower in platelets prepared using automated TACSI method compared to semi-automated [33]. Interestingly, France was the only respondent that routinely used TACSI to produce their whole blood derived platelets (70% of their supply), yet the rate of alloimmunisation for D mismatched platelets observed (1%) appears to be similar to other studies [25]. Conversely, factors in the recipient such as immunosuppression, as well as the frequency and amount of platelets transfused might all influence how many red cells will elicit a response.

Question 8 Do the answers to Questions 5 and 6 apply to all types of platelet, or does this depend on the level of red cell contamination?

All responding sites applied policies for RhD-negative platelet provision and mitigation of alloimmunisation to RhD irrespective of the type of platelets or red cell contamination.

Question 9 Do you have a system for routinely capturing cases of alloimmunisation to RhD due to platelet transfusions, in haemovigilance schemes for example?

5/15 respondents mention some form of reporting scheme where such cases may be captured. In addition, one country has recommendations on testing 1–4 months following RhD incompatible transfusion, and one has done a two-year analysis where RhD-positive platelets were given to RhD recipients without anti-D, showing 1.4% rate of alloimmunisation.

CONCLUSIONS

In summary, this international forum highlights some of the different approaches currently taken to provide ABO and RhD compatible platelets. It was notable that there was a significant difference in whether respondents prioritize major or minor ABO compatibility of platelets. This probably reflects the uncertainties with regards to the clinical consequences of ABO incompatible platelet transfusion. Approximately half of respondents prioritized the ABO group of the platelets themselves, indicating that the rationale was that the risk of a HTR due to minor incompatibility was reduced by the use of PAS and/or testing for high titre anti-A/B. This appears to be a shift in thinking with blood providers moving to platelets in PAS/introduction of testing. The risk of a HTR due to ABO incompatible platelets was estimated as being between 1:600,000 to 1:2 million, and several respondents noted that no fatal cases had been observed since the introduction of routine testing for high titre anti-A/B. Risk assessments based on large data sets are needed to fully understand how much PAS reduces the risk of HTR from ABO minor incompatible platelet transfusion.

For RhD, most respondents prioritized RhD-negative platelet provision for RhD-negative females of child-bearing potential, and most either gave or recommended the use of prophylactic anti-D if RhD-positive platelets were transfused to this group. Only two respondents considered whether recipients were immunosuppressed in the decision to give prophylactic anti-D, as these patients are less likely to form alloantibodies to mis-matched platelets. Most respondents visually assess the level of red cell contamination of platelets, with varying cut offs for maximal acceptable levels. However, these are all above the level likely to be capable of causing alloimmunisation to RhD in RhD-negative individuals who are immunocompetent. Studies assessing whether a reduction in intact red cells and/or microparticles will reduce the risk of alloimmunisation are needed to fully understand whether such an approach may be beneficial.

ORCID

Rounak Dubey https://orcid.org/0000-0002-9467-5759
Magnus Jöud https://orcid.org/0000-0002-4734-0040
Joan Cid https://orcid.org/0000-0001-5445-4508
Miquel Lozano https://orcid.org/0000-0003-2593-833X
Peter Flanagan https://orcid.org/0000-0001-5270-3910
Gwen Clarke https://orcid.org/0000-0001-7738-3378

Salwa Hindawi https://orcid.org/0000-0003-3804-5434
Jose Mauro Kutner https://orcid.org/0000-0003-3784-6731

REFERENCES

1. Triulzi DJ, Assmann SF, Strauss RG, Ness PM, Hess JR, Kaufman RM, et al. The impact of platelet transfusion characteristics on posttransfusion platelet increments and clinical bleeding in patients with hypoproliferative thrombocytopenia. Blood. 2012;119:5553–62.
2. Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med. 2010;362:600–13.
3. Lozano M, Heddle N, Williamson LM, Wang G, AuBuchon JP, Dumont LJ, et al. Practices associated with ABO-incompatible platelet transfusions: a BEST collaborative international survey: PLATELET TRANSFUSION AND ABO INCOMPATIBILITY. Transfusion 2010;50:1743–8.
4. Culibrk B, Stone E, Levin E, Weiss S, Serrano K, Devine DV. Application of the ADVIA cerebrospinal fluid assay to count residual red blood cells in blood components: automated counting of residual red blood cells. Vox Sang. 2012;103:186–93.
5. Cid J, Yazer MH, Lozano M. Platelet transfusion and respecting patient D type. Curr Opin Hematol. 2015;22:540–6.
6. Shehata N, Tinmouth A, Naglie G, Freedman J, Wilson K. ABO-identical versus nonidentical platelet transfusion: a systematic review. Transfusion 2009;49:2442–53.
7. Gottschall J, Wu Y, Triulzi D, Kleinman S, Strauss R, Zimrin AB, et al. The epidemiology of platelet transfusions: an analysis of platelet use at 12 US hospitals. Transfusion 2020;60:46–53.
8. Sprogøe U, Yazer MH, Rasmussen MH, Antonsen B, Bistrup C, Assing K. Minimal variation in anti-a and -B titers among healthy volunteers over time: implications for the use of out-of-group blood components. J Trauma Acute Care Surg. 2017;82:S87–90.
9. Harm SK, Yazer MH, Bub CB, Cohn CS, Jacob EK, Kutner JM, et al. Seasonal variability is not observed in the rates of high anti-a and anti-B titers in plasma, apheresis platelet, and whole blood units tested by different methods. Transfusion 2019;59:762–7.
10. Carr R, Hutton JL, Jenkins JA, Lucas GF, Amphlett NW. Transfusion of ABO-mismatched platelets leads to early platelet refractoriness. Br J Haematol. 1990;75:408–13.
11. Malvik N, Leon J, Schlueter AJ, Wu C, Knudson CM. ABO-incompatible platelets are associated with increased transfusion reaction rates. Transfusion 2020;60:285–93.
12. Blumberg N, Refaai M, Heal J. ABO matching of platelet transfusions - "start making sense". Blood Transfus. 2015;13:347–50.
13. Zaffuto BJ, Conley GW, Connolly GC, Henrichs KF, Francis CW, Heal JM, et al. ABO-immune complex formation and impact on platelet function, red cell structural integrity and haemostasis: an *in vitro* model of ABO non-identical transfusion. Vox Sang. 2016;110:219–26.
14. Hess JR, Trachtenberg FL, Assmann SF, Triulzi DJ, Kaufman RM, Strauss RG, et al. Clinical and laboratory correlates of platelet alloimmunization and refractoriness in the PLADO trial. Vox Sang. 2016;111:281–91.
15. Kaufman RM, Assmann SF, Triulzi DJ, Strauss RG, Ness P, Granger S, et al. Transfusion-related adverse events in the platelet dose study: PLT transfusion adverse events. Transfusion 2015;55:144–53.
16. Nellis ME, Goel R, Karam O, Cushing MM, Davis PJ, Steiner ME, et al. Effects of ABO matching of platelet transfusions in critically ill children*. Pediatr Crit Care Med. 2019;20:e61–9.
17. Food & Drug Adminstration. Transfusion/Donation Fatalities [Internet]. [cited 2021 Feb 2]. US Food and Drug Administration. Transfusion/Donation Fatalities. https://www.fda.gov/vaccines-blood-biologics/report-problemcenter-biologics-evaluation-research/transfusiondonation-fatalities
18. Karafin MS, Blagg L, Tobian AAR, King KE, Ness PM, Savage WJ. ABO antibody titers are not predictive of hemolytic reactions due to plasma-incompatible platelet transfusions: high-titer plasma-incompatible AP transfusions. Transfusion 2012;52:2087–93.
19. Serious Hazards of Transfusion Annual Reports 1996–2019 [Internet]. [cited 2021 Feb 2]. https://www.shotuk.org/shot-reports/
20. Noël A. Anti-A isoagglutinins and pneumococcal vaccine. Lancet. 1981;318:687–8.
21. Wolfram W, KMT S, Binder CJ, Eibl-Musil N, Wolf HM, Fischer MB. Pneumococcal polysaccharide vaccination elicits IgG anti-a/B blood group antibodies in healthy individuals and patients with type I diabetes mellitus. Front Immunol [Internet]. 2016;7 [cited 2021 Feb 5]:493.
22. Daniel-Johnson J, Leitman S, Klein H, Alter H, Lee-Stroka A, Scheinberg P, et al. Probiotic-associated high-titer anti-B in a group a platelet donor as a cause of severe hemolytic transfusion reactions. Transfusion 2009;49:1845–9.
23. Berséus O. Effects on the anti-ABO titers of military blood donors from a predeployment vaccination program. J Trauma Acute Care Surg. 2017;82:S91–5.
24. Klein A. Mollison's blood transfusion in clinical medicine. 12th ed. Hoboken, New Jersey: Wiley-Blackwell; 2014.
25. Cid J, Lozano M, Ziman A, West KA, O'Brien KL, Murphy MF, et al. Low frequency of anti-D alloimmunization following D+ platelet transfusion: the anti-D Alloimmunization after D-incompatible platelet transfusions (ADAPT) study. Br J Haematol. 2015;168:598–603.
26. Standards for blood banks and transfusion services. 32nd ed. Bethesda, Maryland: AABB publishing; 2020.
27. Guide to the preparation, use and quality Assurance of Blood Components. 20th ed. Strasbourg, France: Council of Europe Publishing; 2020.
28. Gunson HH, Stratton F, Cooper DG, Rawlinson VI. Primary immunization of Rh-negative volunteers. BMJ. 1970;1:593–5.
29. Jakobowicz R, Williams L, Silberman F. Immunization of Rh-negative volunteers by repeated injections of very small amounts of Rh-positive blood. Vox Sang. 1972;23:376–81.
30. Thibault L, de Grandmont MJ, Cayer M-P, Dussault N, Jacques A, Ducas E, et al. Rhesus D antigenic determinants on residual red blood cells in apheresis and Buffy coat platelet concentrates. Transfus Med Hemother. 2020;47:129–34.
31. Victoria E, Branks M, Masouredis S. Immunoreactivity of the rho(D) antigen in cytoskeleton-free vesicles. Transfusion 1987;27:32–5.
32. Santana JM, Dumont LJ. A flow cytometric method for detection and enumeration of low-level, residual red blood cells in platelets and mononuclear cell products. Transfusion (Paris). 2006;46:966–72.
33. Cavagnetto C, Blanco RA, McKenna H, Willmott L, Aydogdu E, Akinyemi N, et al. Residual red cells in blood components: a multisite study of fully automated enumeration using a hematology analyzer. Transfusion 2020, published ahead of print. https://doi.org/10.1111/trf.16196

Guest Editors
Rebecca Cardigan
NHS Blood & Transplant, Cambridge, UK
Email: rebecca.cardigan@nhsbt.nhs.uk

Helen V. New
NHS Blood & Transplant, London, UK
Email: Helen.new@nhsbt.nhs.uk

Lise Estcourt
NHS Blood & Transplant, Oxford, UK
Email: lise.estcourt@nhsbt.nhs.uk

International Forum Editor
Nancy Dunbar
Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA
Email: Nancy.M.Dunbar@hitchcock.org

How to cite this article: Cardigan R, New HV, Estcourt L, Zhiburt E, Dubey R, Bengtsson J, et al. International Forum on Policies and Practice for Transfusion of ABO and RhD Non-Identical Platelets: Summary. Vox Sang. 2021;1–9.

Received: 26 April 2021 | Accepted: 27 April 2021

DOI: 10.1111/vox.13130

INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on Policies and Practice for Transfusion of ABO and RhD Non-Identical Platelets: Responses

Rebecca Cardigan | Helen V. New | Lise Estcourt | Eugene Zhiburt | Rounak Dubey | Jesper Bengtsson | Magnus Jöud | Carlos Castillo | Joan Cid | Miquel Lozano | Dhana Gounder | Peter Flanagan | Sarah Morley | Gwen Clarke | Dana Devine | Salwa Hindawi | Aqeel AlOtaibi | Carolina Bonnet Bub | Jose Mauro Kutner | Toshiyuki Ikeda | Naoko Goto | Hitoshi Okazaki | Magali J. Fontaine | Jeremiah Pasion | Linda Song | Tom Latham | Jean-Louis Kerkhoffs | Masja de Haas | Jaap Jan Zwaginga | Birgit S. Gathof | Katharina Ommer | France Pirenne | Michel Raba | Anne Francois | James Daly | Tanya Powley | Nancy Dunbar

RUSSIA

Eugene Zhiburt

Question 1

We have apheresis (70%) and whole blood derived (buffy coat) (30%). Each pool is derived from four to five donors. Whole blood processing is mostly manual.

Platelets are suspended in plasma or in SSP+ [1].

Platelets for neonates/infant transfusion have the same specification as those for adults but more often are irradiated [2].

Question 2

According to our national/governmental guide:

- Transfusion of platelet concentrate non-identical in the ABO system is allowed, obtained using an additional solution;
- Transfusion of whole-blood derived platelet concentrates from a blood units of group O or apheresis platelet concentrates AB group to a recipient with any blood group is allowed.
- 21% platelet transfusions are ABO non-identical.

Question 3

Among those mentioned strategies only storage of platelets in PAS is used to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility.

Plasma transfusion of the AB group to the recipient with any blood group is allowed

Question 4

I did not observe cases of haemolytic transfusion reactions to platelets, and no such cases reported in national haemovigilance scheme.

Question 5

According our national/governmental guide, the compatibility of the donor and adult recipient in terms of red blood cell antigens D, C, c, E, e, K is not taken into account when transfusing platelet concentrates obtained using apheresis or using an additional solution or pathogen-reduced platelet concentrate.

Question 6

We do not have any guide for preventive treatment with anti-D.

Question 7

We do not routinely assess the level of red cell contamination of platelets.

Question 8

During last 20 years I did not see issued 'red' platelets.

Question 9

We do not have a system for routinely capturing cases of alloimmunization to RhD due to platelet transfusions.

REFERENCES

1. Gubanova MN, Ayupova RF, Zhiburt EB. Polymorphism of collection, transfusion and effectiveness of donor platelets concentrates. Anesteziol Reanimatol. 2017;62:77–9.
2. Bruun MT, Yazer MH, Spinella PC, Titlestad K, Lozano M, Delaney M, et al. Vox Sanguinis international forum on paediatric indications for blood component transfusion. Vox Sang. 2019;114:e36–e90.

Eugene Zhiburt
Pirogov National Medical Surgical Centre, Moscow, Russia
Email: ezhiburt@yandex.ru

INDIA

Rounak Dubey

Question 1

Platelets products:

- Both apheresis and whole blood-derived platelets are supplied by our centre. Whole blood-derived platelets are prepared from routine blood donations while apheresis platelets are collected only after the demand is received. The PRP method is used for preparing platelets from whole blood donations. The pooling of platelets is not done. The processing is by manual/semi-automated methods.
- Around 90% of the demand is met by whole blood-derived platelets while the remaining 10% need the apheresis product.
- Platelets are suspended in plasma. Platelet additive solutions are not used.
- Same platelets are used for transfusion in adults and neonates/infants, but the volume of required platelets varies as per weight of the infant.

Question 2

The recommendations of the local Hospital Transfusion Committee (HTC) are used as the guiding policy for transfusion of ABO non-identical platelets. The standard practice is to give ABO identical platelets, followed by ABO (plasma) compatible platelets (avoiding minor incompatibility), only after ensuring there is no RBC contamination. Out of group platelet transfusions are mostly an emergency requirement and account for nearly 5% of total platelets issued.

Question 3

Strategies to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility:

- For planned/non-emergency requirements, only ABO identical platelets are provided. ABO non-identical platelets are only issued after the treating clinician has been informed and the demand form mentions that ABO non-identical platelets may be provided as an emergency measure. Platelets of the blood group 'O' are avoided while giving to other blood groups.
- Volume reduction is done only for apheresis platelets (if the situation permits adequate time), the final product is around 100–110 ml.
- Platelets are not stored in PAS.
- Testing of donor/product for anti-A/-B.
  a. The donor or the product is not tested for anti-A/-B titres. Blood grouping and antibody identification are performed on the donor samples.
  b. Not Applicable
  c. No.
  d. Do you also test plasma/whole blood for transfusion?
  Answer: Yes
  e. There is no written policy, but the guiding principle is similar to transfusion of ABO-incompatible platelets. In practice, ABO-incompatible plasma is rarely issued as the group identical plasma is usually available. Cryoprecipitates are more commonly issued across the ABO blood group.

Question 4

The haemolytic transfusion reactions due to platelets are rare and no such reaction has been reported in past 1 year at the centre. The fact that it is mostly carried out during an emergency and often subclinical, makes it less likely to be reported or observed. There is a provision to report them to the National Hemovigilance Programme of India while submitting the monthly haemovigilance report.

Question 5

There are no national/regional clinical polices with respect of transfusion of RhD-negative platelets. Females in the child-bearing age group are specifically considered for RhD-negative platelets. If available, RhD-negative platelets are given and in case apheresis is planned, Rh Negative donors are preferred.

Question 6

The platelets which do not have any visible RBC contamination are given. The decision to administer anti-D rests with the treating clinician and varies from one case to another. In case of obstetric patients, anti-D is more commonly given prophylactically.

Question 7

A assessing the level of red cell contamination of platelets:

- The level of red cell contamination of platelets is routinely assessed by visual examination of all platelets to ensure there is no pink/red discolouration. The absence of pink/red discolouration is taken as the RBC levels to be below 0.5 ml in the bag (which corresponds to a count of 5×10^9 RBCs).
- This information is not put on the label.

Question 8

Yes, the answers apply to all types of platelets. If there is any visible red cell contamination, the RhD-positive platelets are not issued to such patients.

Question 9

There is no specific provision for capturing cases of alloimmunization in such patients. A common transfusion reaction reporting form is issued along with all the blood products and any transfusion-associated complication may be reported back to the blood centre.

Rounak Dubey
Santokba Durlabhji Memorial Hospital, Jaipur, India
Email: rounakdubey@gmail.com

SWEDEN

Jesper Bengtsson & Magnus Jöud

Question 1

We supply platelet components (PCs) derived both from whole blood and apheresis. In 2019, 7480 PCs were transfused in our region. The majority, 93.2%, were whole blood-derived (pooled) PCs and 6.8% were apheresis PCs. We normally only produce group O and group A PCs.

Pooled PCs are produced via the Reveos automated blood processing system and interim platelet units (IPUs). We have two platelet production lines, fresh blood (FB) and overnight (ON). Typically four IPUs are pooled to form a platelet concentrate, on occasion three, five or six IPUs can be used for a pooled platelet concentrate. The pooled IPUs are suspended in platelet additive solution, T-PAS+ (PAS-E). Pooled PCs produced by Reveos contain about 40% plasma.

Apheresis PCs are collected on Trima Accel system from (preferably) male donors, generally blood group O and A. The apheresis PCs are also suspended in T-PAS+ with a plasma content of 35%.

Platelets for neonatal or infant use have the same specifications as those for adults, with the exception that split units from the same donor are generally reserved for the patient in order to minimize donor exposure.

Question 2

We have local policies that outline how ABO non-identical PCs are allowed to be transfused. If ABO identical platelets are not available, we use ABO minor incompatible or, to a lesser extent, ABO major incompatible PCs. All group A platelet apheresis donors are phenotyped for A1 and PCs from group A_2 donors are considered to be functionally group O.

In 2019, 21.7% of platelet transfusions were ABO non-identical: 18.1% minor, 3.2% major and 0.4% bi-directional incompatible. Furthermore, 10.7% of all PCs were transfused to patients with undeterminable ABO-group following ABO non-identical allogeneic haematopoietic stem cell transplantation (aHSCT).

Question 3

Both whole blood- and apheresis-derived ABO minor incompatible PCs are used but anti-A/-B is only considered for apheresis PCs. High-titre apheresis PCs can also be washed to remove ABO-incompatible plasma. This is sometimes necessary to provide compatible platelets to HLA-immunized patients when the matching apheresis donor has a high anti-A/-B titre. All platelets are stored in PAS-E.

At present, we measure anti-A and/or -B titres only once in platelet apheresis donors. Depending on the titre, all PCs derived from the donor are regarded as being low or high titre and PCs labelled

accordingly. Anti-A and -B titres are measured in anti-IgG and -NaCl gel cards, representing IgG and IgM titres, respectively. IgM titres ≥64 and IgG titres ≥256 are considered high.

We do not use ABO-incompatible plasma, cryoprecipitate or whole blood for transfusion.

Question 4

At our centre we have not seen any haemolytic transfusion reactions due to anti-A/-B in PCs, at least not the past 15 years.

In the last decade, only one incident of an acute haemolytic transfusion reaction due to an ABO minor incompatible platelet transfusion has been reported to the Swedish national haemovigilance system. This reaction was reported from another blood centre in 2011, with no further details available.

Question 5

RhD-negative PCs are indicated for all RhD-negative patients according to our local policy, with some exceptions. When the supply of RhD-negative platelets is insufficient RhD-positive PCs are also transfused to RhD-negative patients unless they are children (<18 years) or women of childbearing age (<50 years). If the supply of RhD-negative platelets is very low, a laboratory physician can be consulted and approve the selection of RhD-positive platelets to RhD-negative children and women of childbearing age. Following aHSCT with RhD mismatch between donor and recipient, RhD-negative PCs are transfused if available. When multiple PCs are ordered due to trauma or major bleeding, our policy allows for any platelet unit to be transfused regardless of the patient RhD phenotype, age and gender.

In 2019, 4.9% of all PCs were RhD-positive PCs transfused to RhD-negative patients. No RhD-positive PC was transfused to a RhD-negative child and only 9 units were given to three different RhD-negative women of childbearing age. Two out of these women had been treated with aHSCT with graft from an RhD-mismatched donor and the third was a patient undergoing sex reassignment therapy.

Question 6

For most RhD-negative patients, no steps are taken reduce the risk of alloimmunization following the transfusion of RhD-positive platelets. However, if RhD-positive platelets are given to children or women of childbearing age, we recommend that patients are given anti-D prophylactically.

Question 7

We do not measure red cell content in PCs on a routine basis. The IPUs are visually inspected after production, if they appear reddish they are generally discarded. Hence, there is no need to label PCs.

Question 8

All types of PCs are considered equal in terms of red cell contamination.

Question 9

No, we do not have a systematic follow-up to capture RhD immunization following the transfusion of RhD-positive platelets. We consider the risk of immunization to be low.

Jesper Bengtsson
Email: jesper.bengtsson@skane.se

Magnus Jöud
Skånes Universitetssjukhus Lund, Lund, Sweden
Email: magnus.joud@skane.se

SPAIN

Carlos Castillo, Joan Cid & Miquel Lozano

Question 1

Platelet components are supplied by Blood and Tissue Bank (BST is the Catalan acronym). Blood component preparation is automated with Reveos devices and the majority (95%) of platelet components are obtained with this method (interim platelet unit, IPU) [1,2]. The minimum acceptable platelet content is 2.5×10^{11} per pool after mixing four or five IPUs with platelet additive solution (PAS). The final result is that platelets are stored in plasma/PAS C in a proportion 35%/65%.

Apheresis platelets (5%) are used only for special purposes (e.g., refractoriness, HPA-1 negative supply) and, occasionally, when there are not enough platelets from whole blood.

Platelets for neonate/infant transfusion are obtained after pooling three IPUs obtained from three CMV-negative donors. The minimum acceptable platelet content is 1.5×10^{11} per pool.

Question 2

We do not have national/local clinical policies with respect to transfusion of ABO non-identical platelets. We are not aware of how many platelet transfusions are ABO non-identical, but we prioritize major compatibility because our platelet components are suspended in plasma/PAS C.

Question 3

We are strict about ABO compatibility only when there is a visible red blood cell contamination in the platelet product, which is very infrequent. The risk of haemolytic transfusion reaction because of minor ABO-incompatibility is mitigated because platelet components are suspended in plasma/PAS C. Anti-A/-B is not routinely tested in our donors [3].

We always apply ABO compatibility in plasma transfusion but not with cryoprecipitate.

Question 4

We have not observed any case of haemolytic transfusion reactions after platelet transfusion, at least in the last 30 years. As it is the case with every blood component, any haemolytic transfusion reaction should be reported to the national haemovigilance register.

Question 5

We do not have national/regional clinical policies with respect to transfusion of RhD-negative platelets. RhD-negative platelets are indicated for women with childbearing potential.

Question 6

The administration of anti-D prophylactically is recommended for women <50 years, in case of platelets red blood cells contaminated transfusion.

Question 7

The presence of red blood cells in platelet components is assessed by the quality control plan performed by the Blood and Tissue Bank [4]. Flow cytometry is used to quantify the amount of RBCs in platelet components. There is no upper limit considered acceptable to issue platelets. However, reddish coloured platelets bags are not issued.

Question 8

The answers to Questions 5 and 6 do not depend on the level of RBC contamination.

Question 9

As patients who need platelet transfusion are usually on chronic RBC support, any alloimmunization would be detected in pretransfusion testing. We do not purposely search for platelet transfusion-induced RhD alloimmunization.

REFERENCES

1. Cid J. Automation in blood component preparation methods. In: Blajchman M, Cid J, Lozano M, editors. Blood component preparation: from benchtop to bedside. Bethesda, MD: AABB Press; 2011. p. 271–86.
2. Cid J, Magnano L, Lozano M. Automation of blood component preparation from whole blood collections. Vox Sang. 2014;107:10–8.
3. Lozano M, Cid J. The clinical implications of platelet transfusions associated with ABO or Rh(D) incompatibility. Transfus Med Rev. 2003;17:57–68.
4. Cid J, Lozano M. Risk of Rh(D) alloimmunization after transfusion of platelets from D+ donors to D- recipients. Transfusion. 2005;45:453–4.

Carlos Castillo
Hospital Clinic de Barcelona, Barcelona, Spain
Email: cacastillo@clinic.cat

Joan Cid
Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain
Email: jcid@clinic.cat

Miquel Lozano
Hospital Clinic de Barcelona, Barcelona, Spain
Email: mlozano@clinic.cat

NEW ZEALAND

Dhana Gounder, Peter Flanagan & Sarah Morley

Question 1

We supply both apheresis and manually processed whole blood derived pooled buffy coat platelets in a proportion of approximately 30%–70%. The pooled platelets are derived from four donations. A small percentage of the apheresis platelets are collected in plasma which is for transfusion in neonates and infants (2.60%). The rest of the apheresis and pooled components are suspended in a mixture of plasma (30%–40%) and SSP+ platelet additive solution (60%–70%). We are currently in the process of seeking regulatory approval to move to PAS platelets for neonates and infants as well.

Question 2

All hospital blood banks in New Zealand follow a common protocol that encourages use of ABO identical platelets whenever possible. In New Zealand we predominantly collect Group A and O platelets so a majority of B and AB patients will receive out of group transfusions. From June 2019 to May 2020 22.52% non-identical platelets were issued. Where ABO identical platelets are not available our policy gives priority to avoiding major incompatibility.

Question 3

The use of PAS has been shown to significantly reduce IgM and IgG titres [1]. While it is not clear whether this will actually reduce the risk of haemolysis due to ABO isoagglutinins our haemovigilance reporting which began in 2005 has not had any reports since 2012. We started manufacturing PAS platelets in 2010 (pooled) and 2011 (apheresis).

Haemolysin screening is done on every donation. Ours is a semi-automated micro titre plate method using A_1B cells. The test detects IgM and IgG with the wells being visually checked for haemolysis and a >50% reduction of the cell button to call it haemolysin positive. Similar testing is done on donors for all donated components. While our policy requires that plasma and cryoprecipitate are compatible with the patient's red cells, we have guidelines in place for appropriate selection of incompatible plasma and cryoprecipitate.

Question 4

Since commencement of haemovigilance reporting in 2005 we have had six reports of acute intravascular haemolysis all due to group O platelets. We have not had any reports since 2012.

Question 5

There is a national policy that prioritizes the transfusion of RhD-negative platelets to:

- RhD-negative females of less than 55 years (including female children) who require platelet support for trauma or surgery, that is, where the requirement for support is short lived.
- RhD-negative females less than 55 years (including female children) requiring repeated platelet support for non-malignant conditions where future pregnancies are possible.
- Patients with haematological malignancies and other patients requiring long-term platelet support and RhD-negative children depending on availability.
- Other RhD-negative patients who require short-term platelet support depending on availability.

Question 6

For female patients less than 55 years and young male children who receive RhD-positive platelets RhD immunoglobulin (250 IU) is administered as standard treatment. Female patients of less than 55 years with haematological malignancies and other conditions requiring long term platelet support the risk of sensitisation to anti-D is lessened due to immunosuppressive effects of treatment and/or the underlying condition. In these patients RhD immunoglobulin is considered with decision made by the patient treating clinician.

Question 7

We do not routinely assess the level of red cell contamination of platelets. Our blood service did a recent analysis of red cell contamination indicating levels of 0.078 and 0.012 ml for PAS pooled and PAS apheresis platelets, respectively.

Question 8

The level of red cell contamination is not taken into consideration when transfusing RhD-positive platelets.

Question 9

We do not capture cases of alloimmunization to RhD due to platelet transfusions in our haemovigilance reporting. However, a 2-year (1 May 2010–29 April 2012) analysis done where RhD-positive platelets were transfused to RhD-negative recipients and anti-D prophylaxis was not used the frequency of D alloimmunization was 1.4%. This is very similar to a more recent large study reflecting the experience of 11 centres around the world which showed a frequency of 1.44% [2].

REFERENCES

1. Pagano MB, Katchatag BL, Khoobyari S, van Gerwen M, Sen N, Rebecca Haley N, et al. Evaluating safety and cost-effectiveness of platelets stored in additive solution (PAS-F) as a hemolysis risk mitigation strategy. Transfusion. 2019;59:1246–51.
2. Cid J, Lozano M, Ziman A, West KA, O'Brien KL, Murphy MF, et al. Low incidence of anti-D alloimunization following D+ platelet transfusion: the anti-D Alloimmunization after D-incompatible platelet transfusions (ADAPT) study. Br J Haematol. 2015;168:598–603.

Dhana Gounder
New Zealand Blood Service, Auckland, New Zealand
Email: dhana.gounder@nzblood.co.nz

Peter Flanagan
New Zealand Blood Service, Wellington, New Zealand
Email: peter.flanagan@nzblood.co.nz

Sarah Morley
New Zealand Blood Service, Auckland, New Zealand
Email: sarah.morley@nzblood.co.nz

CANADA

Gwen Clarke & Dana Devine

Question 1

Platelets products:

- Whole blood-derived platelets are prepared by the buffy coat method using a pool of four donors. Platelet pool production is performed using semi-automated extractors to isolate buffy coats and to extract after the second spin. Buffy coat pooling is done manually using the train method. Plateletpheresis is used to collect HLA-matched platelets and to supplement whole blood-derived platelet inventory.
- Approximately 75% of platelet doses are derived from whole blood donations and the remainder from plateletpheresis.
- Both buffy coat and apheresis platelets are presently suspended in plasma although implementation of PAS (SSP+) initially for buffy coat platelets is anticipated in the next months.
- Platelets for neonates/infants have the same specifications as those produced for adults.

Question 2

Canadian national standards (CSA Z902-20) state 'The donor plasma in platelets should be ABO compatible with the recipient's red cells. A policy shall be in place concerning group substitution when compatible platelets are not available'. Local polices vary but most try to provide group specific or group compatible with titration of anti-A and -B or plasma reduction used to mitigate risk of haemolysis when/if the plasma suspending the platelets is incompatible with recipient red cells.

The percentage is variable across locations/provinces; overall approximately 20% of platelet transfusions are to a non-ABO identical recipient.

For non-identical platelet transfusion, the priority is the compatibility of the donor plasma with recipient red cells.

Question 3

Strategies to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility:

- Some hospitals will volume reduce when transfusions of non-identical/ABO incompatible platelets are required and the isohaemagglutinin titre is greater than their acceptable cut off or is unknown. This is especially true if a non-identical/incompatible platelet will be used in a paediatric or neonatal patient. For neonates at one reporting site 50 ml of a buffy coat pooled platelet component is concentrated to 20 ml. At another site the whole pooled platelet unit is concentrated to 20–30 ml with 100 ml of saline added back.
- PAS is not currently used in Canada, although its implementation is planned.
- This is done by most large hospital transfusion sites.
  a. Currently the product is tested by the transfusing hospital upon receipt from the blood supplier. There is a project underway at Canadian Blood Services to begin testing all donors on each donation (planned implementation in 2022).
  b. Hospital sites use a tube technique—supernatant plasma is diluted in saline to 1:100 or 1:50 then tested versus A1 and/or B reagent cells (depending on the group of the platelet). Sites variously use a titre of >50 or >100 as the cut off above which they would not use the platelet unit for non-ABO identical recipients.
  c. As the product is tested, not the donor, the testing is repeated each time a product is received at the transfusing facility.
  d. Units are not labelled with the specific titre; instead a tag or label is applied indicating that the unit is positive or negative for high titre anti-A or -B and suitable for patients of all groups (low titre) or only for ABO group-specific recipients (high titre).
  e. Isohemagglutinin titres are not tested in plasma; whole blood for transfusion is not currently available in Canada.
  f. Some sites have local policies advocating use of group A plasma (with anti-B titres generally not known) for patients of unknown blood groups in a massive haemorrhage setting. For plasma transfusion, apart from this circumstance, group-specific plasma is typically provided.
- Cryoprecipitate transfusion does not require blood group specificity.
- CSA Z902-20 requirements are as follows:
  - Standard 10.7.6 states 'Plasma selected for transfusion shall be ABO compatible with the recipient's red blood cells but does not require compatibility testing. A policy shall be in place concerning group substitution when compatible plasma is not available'.
  - Standard 10.7.7 states 'A policy shall be in place concerning ABO compatibility of cryoprecipitate components'. Note: All recipients may be transfused with any ABO group of cryoprecipitate.

Question 4

Since 2017, there have been two reports of ABO hemolytic transfusion reactions related to ABO-incompatible platelet transfusion. One was a group B patient who received a group A platelet pool; the second was a group AB patient who received a group O platelet.

Question 5

Platelets are matched for RhD as routine practice in most institutions if ABO/Rh compatible platelets are available. There is no national clinical policy concerning transfusion of RhD-negative platelets. Canadian National Standards (CSA Z902-20) state only that there must be a policy regarding management of RhD-negative recipients of RhD-positive

blood components. Specifically, standard 11.9.7 states 'Each transfusion service shall have a policy for the management of RhD negative recipients who receive blood components containing RhD positive red cells'.

Hospitals would endeavour to provide only platelet components from RhD-negative donors to female patients <45 years old who are RhD negative. In some transfusion services this allocation of only RhD-negative platelets to RhD-negative recipients would extend to all paediatric patients (male and female).

Question 6

Use of anti-D may be considered if RhD-positive platelets are transfused to RhD-negative female children or women of child-bearing age. Anti-D treatment should be performed within 72 h of transfusion of RhD-positive platelets to an RhD-negative recipient. While local practices vary, RhIG (anti D) is given prophylactically by most transfusion services to female patients who are <45 years old, known to be RhD negative and who have received one or more platelet pools or apheresis platelets obtained from RhD-positive donors. For some transfusion services RhIG would also be given to paediatric patients, male or female who have received platelet pools from RhD-positive donors.

Question 7

There is currently no standard in Canada for the allowable amount of RBC contamination in platelet products. All products are subjected to visual assessment prior to release. Red cell contamination is assessed against a visual assessment guide and platelets are not released if marked in red. This visual assessment guide is also available to hospital customers. The level of RBCs is formally assessed whenever production practices are changed; assessments are made using flow cytometry-based residual RBC assays. Residual RBC levels are not routinely measured in production.

Question 8

Use of anti-D prophylaxis is used at the discretion of the treating physicians and in accordance with local policy but generally applies to all types of platelets, regardless of whether visible red cell contamination is present.

Question 9

Alloimmunization to RhD arising from platelet transfusions may be reported to the blood supplier or to the Canadian hemovigilance system, the Transfusion Transmitted Injuries Surveillance System, operated by the Public Health Agency of Canada. The reporting is voluntary and under reporting is likely.

Gwen Clarke
Canadian Blood Services, Edmonton, AB, Canada
Email: gwen.clarke@blood.ca

Dana V. Devine
Canadian Blood Services, Vancouver, BC, Canada
Email: dana.devine@blood.ca

SAUDI ARABIA

Salwa Hindawi & Aqeel Al Otaibi

INTRODUCTION

The blood transfusion services in Saudi Arabia are fragmented hospital-based blood banks. National standards for hospitals including blood banks are available and obligatory to be followed by all hospitals (Central Board of Accreditation and Healthcare Institutions, CBAHI), and international standards (American Association of Blood Banks, AABB) are voluntarily followed as an international accreditation body for blood banks [1,2].

Although compliance of the National wide standard is expected, the policy and practice may differ from facility to facility with the reference of national and international accreditation bodies to make sure of not deviating from the national standards. Information is collected through two different facilities, which have both accreditations national and international (AABB), as an example of blood banks in Saudi Arabia.

Question 1

At both facilities, we are in practice of collecting both apheresis and whole blood-derived platelets (PRP only) Whole blood donation processing is through Reveos with the pool of 4–6 units to reach not less than 300 yield (one therapeutic dose 3×10^{11}) and Apheresis platelet with the single dose as an adult dose. All platelets concentrates are leucodepleted. Other hospitals in the country also may do Apheresis and Manual PRP or Automated PRP. Proportion of Apheresis and WBDP is 10%–20%/90%–80%, respectively (it could be more in certain hospitals). Storage media of both the procedures are 100% Plasma.

For neonates/infant transfusions we have volume reduced and not like adults, neonatal patients must receive ABO-compatible plasma.

Question 2

There is a local policy regarding platelet transfusion; Platelet transfusions should be ABO identical, if it is unavailable should be ABO

compatible for adults. Always issue Rh-negative platelets to Rh-negative patients especially to females of childbearing age. If none is available and there is urgent need to transfuse Rh-positive platelets, inform physician or nurse of the situation and suggest the administration RH immunoglobulin as per hospital policy. For neonates, they must receive ABO-compatible plasma or volume reduced in case of compatible group use. In case of emergency transfusions or for platelet refractoriness and when ABO identical or compatible units are not available, we may issue other blood groups as per the selection priorities [1,3,4].

The percentage of ABO non-identical Platelet transfusions is around 20%.

As a policy of the blood transfusion services, the use of Rh-positive red cell-containing components such as platelets concentrate and platelets apheresis to Rh-negative recipients will required a written approval from the physician in-charge and the medical director or delegate should be informed.

Question 3

The strategies/policies are as follows:

- If any patient requires a platelet transfusion and only non-group specific or non-group compatible is available an approval from the physician in charge or haematologist on call (or BTS medical director) should be obtained.
- Volume reduction of platelets prior to transfusion is in practice and the final plasma volume will be reduced to 30% from the actual volume of the initial units.
- Volume reduction is used for neonatal and for patients suffering ill effects if the whole volume is infused.
- There is no routine testing of donor or final products for anti-A/-B.

Question 4

There are no reported cases noted of haemolytic transfusion reactions due to platelets transfusion, we do have system in place to report and follow up for any transfusion reactions. All adverse reactions are reported on yearly basis to MOH (national body).

Question 5

There is local policy available on transfusions of RhD-negative platelets.

Rh-negative platelet should be issued to Rh-negative patients especially females of childbearing age [1]. If none is available and there is urgent need to transfuse Rh-positive platelets, inform physician or nurse of the situation and suggest the administration of RH immunoglobulin as per local policy.

Question 6

As per local policy to minimize the risk of alloimmunization if RhD-positive platelets are transfused to Rh negative as:

- Anti-D is given to any RhD women of childbearing age receiving RhD-positive platelets.
- The following criteria are considered to be the candidate for Rh Immune Globulin:
  - Pregnant women found to be Rh negative, and no evidence of anti-D is found.
  - Potentially sensitizing episodes during pregnancy as ectopic pregnancies, abortions, ante-partum haemorrhages, foetal deaths, closed abdominal injury and amniocentesis patients etc. Kleihauer test or flowcytometry test should be done within 2 h following any sensitizing episode. Give additional anti-D IgG as required (125 IU per ml of feto maternal haemorrhage).
  - Women who have received antenatal Rh Immune Globulin are still considered candidates for postpartum Rh Immune Globulin therapy.
  - Any Rh-negative patient (with no evidence of anti-D is found) receiving Rh-positive platelets transfusion.

Question 7

As per policy the assessment of level of red cell contamination of platelets done through visual comparison chart by AABB.

- Apparently red cell-contaminated platelets should be <2 ml. If it is more than 2 ml platelets are discarded except if needed due to emergency situation, routine cross match will be performed. Information is shared with the treating physician or hospital receiving the platelets but the product label will not be specified.

Question 8

Same policy will be applied to all types of platelets.

Question 9

There is a local haemovigilance system in place to report any adverse events including any case of alloimmunization to RhD due to platelets transfusion as follows:

Investigation and Management of Adverse Reaction:

- All type of reactions must be reported to the Blood Transfusion Services and all reactions received must be investigated as per our local policy, King Abdulaziz University Hospital, Blood Transfusion Services Local Policies.

Incident Reports:

- All events that fall outside of the existing policies and procedures of the Blood Transfusion Services (Vein to Vein) must be reported to the Blood Transfusion Services in order to ensure that these are captured and investigated and corrective actions are undertaken. These procedures will provide a mechanism to track and trend all events, which will enable the process of continuous quality improvement and patient safety [4].
- There are other hospitals, which do not have systems for reporting such cases.

Salwa Hindawi
Chief Scientific officer of:
Saudi Society of Transfusion Medicine
Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
Email: sihindawi@yahoo.com

Aqeel AlOtaibi
Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia
Email: a.rooq@hotmail.com

REFERENCES

1. American Association of Blood Banks. Technical manual. Bethesda, MD: AABB; 2014.
2. American Association of Blood Banks. Standards for blood banks and transfusion services. Bethesda, MD: AABB; 2018.
3. Saudi Central Board for Accreditation of Healthcare Institutions (CBAHI) National Standards, 2018. https://portal.cbahi.gov.sa/english/about-us/cbahi-at-a-glance.

BRAZIL

Carolina Bonnet Bub & Jose Mauro Kutner

Question 1

Our Hospital supplies both products: apheresis and whole blood products (PRP).

Each pool is typically derived from six donors and our whole blood donations processing is manual. Our proportion of supply is 5% from whole blood (PRP) and 95% from apheresis. Plasma is the storage media in which platelets are suspended.

Neonate and infant transfusions have the same specifications as those for adults; we do not manage different platelet storages.

Question 2

In Brazil there is a national federal policy recommendation to perform haemolysin test for ABO non-identical platelets transfusion. Therefore, platelets with results of total or partial haemolysis should be avoided in non-isogroup transfusions.

Our institution policy requires the performance of isohemaglutinin titre for all platelet units. Only platelets with isohemaglutinin titres bellow 64, should be used for ABO non-identical platelet transfusions. During the year of 2019, our platelets transfusion records indicate that 64% were ABO identical and 36% were ABO non-identical.

Question 3

In order to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility, we tested all platelets donor for anti-A and/or -B by gel card at room temperature (IgM). We use a cut-off titre of 64. The donor/donation is tested for every new product donated. The product is not labelled with the titre, but the inventory report identifies the titre of each platelet unit. We are not a blood centre, just a transfusion service which collects its' own products, so labelling was not considered necessary.

We do not test plasma/whole blood for transfusion.

In our institution we only perform identical ABO plasma and cryoprecipitate transfusions.

Question 4

We have not observed any haemolytic transfusion reactions associated with platelet transfusion in our service in the last many years. Our internal routine requires follow-up of every reported reaction. The Brazilian Ministry of Health has a haemovigilance tool, which is filled and reported by every transfusion service once a month.

Question 5

There is a national policy recommendation referring that RhD-negative female recipients under 45 years of age should preferably transfused with RhD-negative platelets.

Question 6

In our institution anti-D immunoglobulin is given prophylactically to the patients within 48 h after a RhD-positive transfusion, if RhD-negative platelets are not available.

Question 7

We do not use any laboratory test to assess the level of red cell contamination of platelet concentrates. However, a visual inspection is performed to analyse red cell contamination of platelet concentrates. This information is not described on the label because all the platelets units with red cell contamination by visual inspection are discarded.

Question 8

The answers to Questions 5 and 6 apply to all types of platelets.

Question 9

Yes, we have a haemovigilance workflow in our institution that when an immunohaematology altered result is detected, previous results from the patient are rechecked and clinical history is obtained. If it is identified as a case of RhD alloimmunization, a report to the national surveillance tool is made monthly.

Carolina Bonnet Bub
Hospital Israelita Albert Einstein, Sao Paulo SP, Brazil
Email: carolinabub@gmail.com

Jose Mauro Kutner
Hospital Israelita Albert Einstein, Sao Paulo SP, Brazil
Email: jose.kutner@einstein.br

JAPAN

Toshiyuki Ikeda, Naoko Goto & Hitoshi Okazaki

Question 1

Japanese Red Cross Blood Centers (JRCBC) supply only apheresis platelet concentrates (PC) which are suspended in plasma. The JRCBC do not produce PAS-suspended PC but supply 'washed PC' on demand that is washed and suspended in bi-carbonate/ACD solution. For neonates/infants or children, small sized bags are available. The JRCBC provides six different sized bags containing at least 0.2, 0.4, 1.0, 2.0, 3.0, 4.0 $\times$ 10^{11} platelets in 20-, 40-, 100-, 200-, 250-, 250-ml plasma, corresponding to 1, 2, 5, 10, 15, and 20 units PC, respectively.

Question 2

The Japanese guidelines for the appropriate use of blood products recommend the use of ABO-identical platelet transfusion. Except for HLA-matched PC, almost 100% PC that are issued by JRCBC are ABO-identical. In the case of HLA-matched PC, HLA matching can be prioritized to ABO matching, when ABO-identical platelet is not available.

If minor incompatibility is unavoidable for HLA-matched PC, ABO antibody titres are measured and in case of high antibody titres (≥128×), the use of washed PC is recommended. Washed PC is recommended for any children regardless of donor anti-A/-B titre, when ABO minor incompatible platelet is used. The proportion of non-ABO-identical HLA-matched PC issued in Japan was approximately 30%, and the distribution of O-type minor incompatible HLA-matched PC was less than 1.0% in 2017–2018, In the University of Tokyo hospital, a total number of platelets used in 2019 fiscal year was 4183. The number of random donor platelets (always ABO identical as described above) and HLA matched or compatible donor platelets (HLA-PC) was 4072 (97.3%) and 111 (2.7%), respectively. In 111 HLA PCs, 43 platelets were ABO identical, 33 were ABO major mismatched, and 35 were ABO minor mismatched. Consequently, the frequency of ABO no-identical platelet transfusions was 1.6% (68 in 4183 platelets). The number of ABO major mismatch platelet transfusions to the patients after ABO-incompatible haematopoietic stem cell transplantation and type AB non-identical platelets used before the ABO blood types were determined in urgent massive bleeding cases was eliminated from the totalization.

Question 3

Although the transfusion of ABO identical PC is recommended by the Japanese guidelines, non-ABO-identical PC can be selected only in case there is need to prioritize HLA-matching for patients with platelet transfusion refractoriness. Recently, washed PC is also supplied by the JRCBC, for the reduction of allergic reactions or hemolytic reactions.

Presently, neither volume-reduced PC nor PAS-suspended PC are supplied by the JRCBC. These products can be prepared in the hospitals.

When supplying ABO-minor incompatible HLA-matched PC, anti-A/-B titres, measured by the saline test, are confirmed and in case of high antibody titres (≥128×), the hospital is informed by the JRCBC and the use of the product is left to the physician's discretion. Antibody titres are not indicated in the PC label. The titration is performed on every donation for non-ABO-identical HLA-matched PC.

The Japanese guidelines also recommend the use of ABO identical plasma/whole blood, and antibody titration is not performed for these products. The JRCBC do not supply cryoprecipitate, but hospital transfusion department can prepare it from fresh frozen plasma, which is covered by the Japanese medical insurance.

Quite a few hospitals including the University of Tokyo hospital are using type AB cryoprecipitate to facilitate the inventory management, since cryoprecipitate is not available from the JRCBC but must be prepared at an in-hospital transfusion service department. In the Japanese population, the frequency of type AB donor is approximately 10% and more popular compared to the Caucasian population. Some institutes that have a large medical emergency centre and many opportunities for massive transfusion prepare and administrate ABO identical cryoprecipitate.

Question 4

Yes, four cases of haemolytic reaction as a result of the transfusion of non-ABO-identical HLA-matched PC were reported via Japanese Red

Cross Society haemovigilance system between 2010 and 2019. In this decade, 8,198,560 platelets including 76,433 HLA-matched PC were issued. The estimated incidence of haemolytic transfusion reactions to platelet transfusion by the Japanese Red Cross haemovigilance data was one in 2 million platelets and one in 19,108 HLA-matched platelets.

Question 5

We have no national/regional clinical policies on this matter since the frequency of RhD-negative blood type in the Japanese population is only 0.5%. In fact, in all hospitals, RhD-negative platelets are given routinely to RhD-negative patients regardless of their clinical backgrounds.

Question 6

Japanese Red Cross haemovigilance has received no report of this situation. Tokyo University hospital also has no experience of this at least in the last decade. If the patient transfused with RhD-incompatible platelets is fertile or younger female, we will prophylactically use anti-D for her. However, in Japan, this use is off label, since the only available anti-D antibody-drug in Japan is indicated only for RhD-incompatible pregnancy. This off-label use of anti-D is incompletely described in the national clinical policy.

Question 7

Red cell contamination test of platelets is conducted as a quality control at the JRCBC processing lab with the frequency of 10 PCs a year, which is required by the guideline Minimum Requirements for Biological Products. However, the contamination of RBC in PC is so low that it is unenumerable.

Question 8

We apply above described policies to all types of platelets.

Question 9

In case alloimmunization to RhD due to platelet is reported by the hospitals, it will be captured in the Japanese Red Cross hemovigilance system. However, since RhD negative is not common (about 0.5% [1 in 200]) in Japan and RhD-negative PC is always availabe, there were no reported cases of alloimmunization to RhD by platelet components in the last decades.

Toshiyuki Ikeda
University of Tokyo Hospital, Tokyo, Japan
Email: ikedat-ort@h.u-tokyo.ac.jp

Naoko Goto
Japanese Red Cross Society, Tokyo, Japan
Email: n-goto@jrc.or.jp

Hitoshi Okazaki
University of Tokyo Hospital, Tokyo, Japan
Email: okazakih-tky@umin.ac.jp

UNITED STATES

Magali J. Fontaine, Jeremiah Pasion & Linda Song

Question 1

Our Hospital-based Transfusion Service is supplied with apheresis platelet (AP) only products and supports tertiary medical care, a Level 1 trauma centre, adult and paediatric haematopoietic cell and solid organ transplants, as well as adult and paediatric cardiovascular medical and surgical services, and high-risk obstetric and neonatal care. The AP inventory consists primarily of pathogen reduction technology (PRT) treated AP stored in platelet additive solution (PAS) (80%), AP stored in PAS non-PRT (10%), and conventional AP stored in plasma (10%). APs for neonates/infant transfusion have the same specification as those for adults.

Question 2

In order to optimize AP inventory and to prevent AP outdating, our process does not require AP transfusions to be ABO identical. About 42% of platelet transfusions are ABO non-identical. Our Institutional standard operation procedure meets the national AABB Standards for Blood Banks and Transfusion Services with a process in place to regulate the transfusion of ABO-incompatible plasma containing platelets.

Question 3

AP products containing ABO-incompatible plasma are approved, provided AP is suspended in PAS. If ABO-compatible plasma containing AP product or if PAS AP product is not available, AP product would be volume reduced down to 50 ml if time allows (volume reduction requirement is waived if AP transfusion is needed emergently) [1]. Blood products containing ABO-incompatible plasma are not currently tested for anti-A/-B titres. Policies for ABO-incompatible plasma for

cryoprecipitate and conventional plasma are to primarily use ABO-compatible plasma first if available.

Question 4

Despite the steps taken to reduce the risk of haemolytic transfusion reactions to platelet transfusion, such as default to AP in PAS or volume reduced if containing ABO-incompatible plasma, our centre has observed haemolytic reactions to AP in PAS and is now considering testing for antiA/B titres in all APs using an automated gel method [2,3].

Question 5

There is no national or regional policy with respect to transfusion of RhD-negative platelets. Platelets are issued primarily based on expiration date.

Question 6

Patient's physician is contacted and anti-D is proposed to be given prophylactically.

Question 7

The level of red cell contamination of platelets is evaluated by simple visual inspection of the AP product.

Question 8

These measures are applied to all platelet products.

Question 9

We do not have a system in place to capture cases of alloimmunization to RhD due to platelet transfusion.

REFERENCES

1. Fontaine MJ, Mills AM, Weiss S, Hong WJ, Ferrer Z, Dunlap M, et al. Platelet inventory management to reduce ABO incompatible plasma following a sentinel event. Transfusion. 2012;52:2081–5.
2. Fontaine MJ, Webster J, Gomez S, Pham TD, Goodnough LT, Galel SA. How do I implement an automated screen for high-titer ABO antibody as an inventory management tool for ABO plasma-incompatible platelets? Transfusion. 2015;55:2783–9.
3. Pasion J, McCusker M, Shimer L, Desai N, Bryant A, McGann HL, et al. Unrecognized acute hemolytic reaction from an out-of-group HLA compatible platelet transfusion. Transfusion. 2019;59(S3):198A.

Magali J. Fontaine
University of Maryland School of Medicine, Baltimore, MD, USA
Email: mfontaine@som.umaryland.edu

Jeremiah Pasion
University of Maryland Medical Center, Baltimore, MD, USA
Email: jeremia.pasion@umm.edu

Linda Song
University of Maryland Medical Center, Baltimore, MD, USA
Email: linda.song@umm.edu

UNITED KINGDOM

Tom Latham & Rebecca Cardigan

Question 1

Platelets products:

- We supply both apheresis platelets collected by Trima, and buffy coat pools made from four donations (manually).
- About 50% of each.
- Apheresis are currently stored in plasma, buffy coat platelets in 65% PAS (SSP+). We are looking to move our apheresis platelet to PAS in future.
- Platelets for neonates and infants are a different product. The main difference is that these are produced by splitting an adult dose into four and stored in smaller packs. Additionally, donors must have donated within the past 2 years and tested negative for mandatory markers of infection, and they must be CMV seronegative and free from clinically significant irregular blood group antibodies including high titre anti-A and -B as defined below.

Question 2

National guidelines state 'It is acceptable to use ABO incompatible platelets to reduce wastage'. Units tested and negative for high titre haemagglutinins and non-group O platelets are associated with a lower risk of haemolysis. Pooled platelets suspended in PAS would also be expected to reduce this risk. Priority is given to minor incompatibility, that is, that of plasma with recipients red cells [1].

We do not have accurate figures for the number of ABO non-identical transfusions. The preference is to give ABO identical where possible. Recent local audit at one large hospital suggests this is about 25%, but nationally this is probably lower at 10%–15% as many smaller hospitals are less likely to give ABO non-identical platelets. The percentage of transfusions which are ABO non-identical may be higher for neonates as only a limited

range of blood groups are routinely stocked for neonatal platelets.

Question 3

Strategies to mitigate the risk of haemolytic transfusion reactions due to ABO incompatibility:

- No, but ABO identical is given by preference.
- No.
- Yes, but only for pools, not apheresis.
- Testing of donor/product for anti-A/-B.
  a. Every donation is tested for high titre antibodies.
  b. We test for IgM only using a microtitre method on an Olympus PK7300 analyser using A2B cells. Samples are diluted 1/32, we have shown this is equivalent to 1/128 by either manual saline method or gel card.
  c. Each time, that is, every donation.
  d. No, if all donations in the pool test as negative then the product is labelled as HT negative. All other units are regarded as not being 'low titre'.
  e. We test every donation. Plasma is labelled as HT negative if it tests as such. Hospitals can select HT negative units is transfusing out of group. We do not provide whole blood routinely, but that being produced by us for trials is tested and only issued if HT negative.
  f. Yes, although AB plasma products are more commonly given in an unknown group situation in contrast to A platelets and group O plasma is always avoided for non-Group O recipients [2].

Question 4

These are reported to our national hemovigilance scheme (SHOT) and the MHRA our regulators. Prior to 2008 a number of HTR to platelets were reported, mainly group O platelets to non-O recipients. As a result in 2008 the methodology for testing high-titre anti-A/-B in donations was standardized in the UK. Between 2009 and 2019 there have been:

- Six possible HTR from ABO-incompatible platelets
- None fatal
- Two cases were difficult to identify causality as the recipient also received red cells or IvIG
- Of the other four, three were from group O to non-O recipient, one was A to AB. In all four elutions, the relevant antibody from red cells was noted.
- In three of the four the platelet were tested as HT negative, two in paedi patients and one in adult (the latter had a IgM around the level of the HT cut-off, but an IgG which is not routinely tested of around 1/2000)
- If we assume 250,000 platelets issued per year and four cases over 10 years then the frequency is 1:625,000

No cases since 2016 when PAS for pooled PC introduced (only 50% of supply) given the low number of cases per year before that, this could be due to chance.

Question 5

BSH guidelines [1]: RhD-negative girls or women of childbearing potential should receive RhD-negative platelets. If unavailable, RhD-positive platelets can be given with anti-D prophylaxis (1B).

For RhD-negative boys under 18 years of age, those who already have anti-D antibodies, and transfusion-dependant adults, the platelets of choice are RhD negative. RhD-positive platelets should be given if RhD-negative platelets are unavailable or to prevent wastage of RhD-positive components. Anti-D prophylaxis is not required (1B).

Question 6

Anti-D is recommended for females of childbearing potential.

Question 7

Assessing red cell contamination of platelets:

- We do not test platelets routinely, we do a visual inspection based on the use of a visual colour chart. The upper limit is 4×10^9 rbc/L, units above this are discarded.
- This information is not on the label.

Question 8

All types of platelets.

Question 9

Not routinely. Development of anti D in a female of childbearing potential is however reportable to the SHOT haemovigilance scheme and an association with platelets might be uncovered. There have been no reports where platelets have been implicated in this group for at least 10 years.

REFERENCES

1. Estcourt LJ, Birchall J, Allard S, Bassey SJ, Hersey P, Kerr JP, et al. Guidelines for the use of platelet transfusions. Br J Haematol. 2017; 176:365–94.

2. Green L, Bolton-Maggs P, Beattie C, Cardigan R, Kallis Y, Stanworth SJ, et al. British Society of Haematology Guidelines on the spectrum of fresh frozen plasma and cryoprecipitate products: their handling and use in various patient groups in the absence of major bleeding. Br J Haematol. 2018;181:54–67.

Tom Latham
NHSBT, Bristol, UK
Email: tom.latham@nhsbt.nhs.uk

Rebecca Cardigan
NHSBT, Cambridge, UK
Email: Rebecca.cardigan@nhsbt.nhs.uk

THE NETHERLANDS

Jean-Louis Kerkhoffs, Masja de Haas & Jaap Jan Zwaginga

Question 1

In the Netherlands buffy coat platelet concentrates derived from five donors are used as well as apheresis platelets. More than 90% of the used platelet products are buffy coat platelet concentrates. Whole blood donations as well as pooled buffy coats are processed using a semi-automated method (Compomat). All platelet concentrates are suspended in 65% PAS-E/35% plasma. For neonates and infants smaller units are used.

Question 2

In the Dutch transfusion guideline it is advised to select platelets that are compatible for the ABO blood group to avoid major incompatibility. However major ABO-incompatible platelet transfusions are used in less than 5% of the transfusions (mostly patients with blood group O receiving group A platelets due to stock/logistical issues).

Question 3

ABO identical platelet transfusions are generally advised when possible. But this is not always possible. In this respect major incompatible platelets can have less increment and can induce anti-A and -B titres and have additional side effects in this regards. Minor ABO-incompatible platelets can result in positive DATs and donors with high titres also some but also sometimes severe haemolysis. In neonates this is considered critical and lower than 1/128 anti-A or -B titres needed. The use of PAS mitigates these minor incompatible side effects but this is not complete.

- In volume-reduced platelet concentrates, the final volume is 30 ml and for neonates 11 ml, at average, respectively. The use of these so-called hyperconcentrated platelets does not change the ABO compatibility recommendations for product selection. Hyperconcentraties are not frequently used: approximately 200 products per year prepared for adults and 100 per year for newborns.
- We use PAS-E as storage medium, this did not yet change the recommendations regarding ABO compatibility of products.
- Testing of donor/product for anti-A/-B.
  a. We are testing the final product for anti-A/-B, and only for products that are used for transfusion to neonates.
     We are performing direct agglutination in tube testing (saline). The cut-off is 64 (not higher than 1+ agglutination) and 128 should be negative.
  b. All final products used for platelet products for newborns are tested, so every time the donation is tested, at this moment.
  c. No, the product is not labelled with the titre.
  d. We are using solvent-detergent plasma as standard plasma product. If FFPs are transfused, those are not routinely tested for antibody titres. Plasma is always given ABO compatible so never (minor) ABO incompatible.
  e. Minor incompatible plasma is not/never given. (Minor) ABO-incompatible platelets in this respect are sometimes given (see above). In the Netherlands, we are not using cryoprecipitate.

Question 4

RhD haemolytic reactions upon platelet transfusions do sometimes happen, although guidelines advise to give RhD-compatible platelets; for females in the reproductive age (<45 years) RhD-compatible blood platelets are advised or RhIg D prophylaxis (375 IU) should be given in addition to an RhD-incompatible product in this age group. This kind of adverse reactions are reported to our national haemovigilance bureau (TRIP) and in 2018 one platelet product was RhD-incompatible transfused: no anti D developed; in that year no AHTR or delayed HTR by platelets were reported.

Question 5

For RhD-negative females aged <45 years RhD-negative platelets need to be selected (see below).

In product packages prepared in case of massive bleeding; the red blood cell and platelet products are all group O, RhD-negative and units of solvent detergent plasma are group AB.

Question 6

The Dutch transfusion guideline it is advised to select RhD-compatible platelets for transfusion. If you cannot select RhD-negative units for RhD-negative recipients, only in case it concerns women aged below 45 years you need to consider to administer RhIg (375 IU). If it concerns a newborn girl (<3 months of age), one

may choose to omit the RhIg administration because of the low risk of RhD alloimmunization.

Question 7

For every product at processing and release department, there is a visual check on 'red appearance' with for comparison a set of calibrated photographs of platelet concentrates spiked with various levels of red blood cells. The maximum level of red blood cells is 6×10^9/L. This information is not shared on the label. On the label there is a link to the online information on product requirements.

Question 8

The level of red blood cell contamination does not change any of the above-mentioned policies.

Question 9

Alloimmunization caused by transfusion needs to be reported to our national institute of haemovigilance. In most causes a root cause is performed if anti-D is developed after transfusion in RhD-negative recipients (see above).

Jean-Louis Kerkhoffs
Sanquin Blood Bank, Amsterdam, The Netherlands
Email: j.kerkhoffs@sanquin.nl

Masja de Haas
Sanquin Research, Amsterdam, The Netherlands
Email: m.dehaas@sanquin.nl

Jaap Jan Zwaginga,
Sanquin Research, Amsterdam, The Netherlands.
Email: j.j.zwaginga@lumc.nl

GERMANY

Birgit S. Gathof & Katharina Ommer

Question 1

- We supply Apheresis (APC) and whole blood-derived (buffy coat PPC) PLT, pools are from presently five in future four donors, processing is manually.
- The mix is about 70% APC, 30% PPC. APC are the preferred choice for haemato-oncologic patients.
- APC in plasma (in future PAS III), PPC in InterSol.
- PLT for neonates have the same specifications.

Question 2

The national policy [1] recommends ABO-identical PLT transfusion; if not available, major incompatible PLT is preferred, but minor incompatible PLT may be used too according to availability.

Question 3

- In PLT production we target a minimum of residual red cells. PPC are stored in PAS, in near future storage of APC-platelets.

Question 4

In our national haemovigilance system haemolytic reactions would be reported, if they occur. Between 2000 and 2017 a total of 13 cases with fatal haemolytic reaction in 7,726,822 PLT transfusion (about 1:600,000) were reported in our national hemovigilance system at PEI [2]. In over 100,000 PLT transfusions at our institute in the past 20 years no haemolytic reaction was observed.

Question 5

According to the National Guidelines [3] for women in childbearing age and children/juveniles under 14 years RhD-negative PLT are indicated.

Question 6

In case of transfusion RhD-positive PLT to RhD-negative patients anti-D prophylaxis is recommended.

Question 7

Red cell contamination is generally low and routinely determined in each PLT product by automated blood count (Sysmex) and in quality control on a specific amount the monthly production (Facs-Scan); according to the National Guidelines it should be $<3 \times 10^9$/product (in our PLT red cells are normally $<1 \times 10^9$). It is not required to report this is on the label.

Question 8

We do not specify for the different products.

Question 9

As anti-D prophylaxis is in use after D-positive PLT transfusion to RhD-negative patients, formation of anti-D after PLT transfusion has not been considered to be a major problem up to now. It is not routinely reported in the national haemovigilance system. While antibody screening is performed regularly on each patient before transfusion of red cells, it is not required before PLT transfusion.

REFERENCES

1. Bundesaerztekammer. https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/Querschnittsleitlinie_Gesamtdokument-englisch_07032011.pdf
2. Haemovigilanzbericht. https://www.pei.de/SharedDocs/Downloads/DE/newsroom/pflichtberichte/haemovigilanzberichte/haemovigilanz-bericht-2016-2017.html;jsessionid=16172A21CDBD52F3CEFD1BD6A6F090EF.2_cid344?nn=12248480.
3. Bundesaerztekammer. https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/MuE/Richtlinie_Haemotherapie_E_A_2019.pdf

Birgit S. Gathof
University Hospital of Cologne, Cologne, Germany
Email: birgit.gathof@uk-koeln.de

Katharina Ommer
University Hospital of Cologne, Cologne, Germany
Email: katharina.ommer@uk-koeln.de

FRANCE

France Pirenne, Michel Raba & Anne Francois

Question 1

- In France we supply both apheresis and blood derived platelets (buffy coat) obtained through 5 to 8 donors, via automated methods (TACSI). All platelets products are then treated for pathogen inactivation (INTERCEPT).
- Apheresis represents about 30% of the French production of platelets. The other 70% are obtained through whole blood.
- The storage media depends upon the technique used. All platelet production is suspended in PASIII (with 30% of plasma and 70% of storage solution). This ratio is mandatory for pathogen inactivation, which is performed on all PC.
- For neonate and infants, we used the most concentrated products in order to obtain at least 0.5 10^{11} platelets in a volume of 30–40 ml. All patients are transfused with apheresis platelets.

Question 2

In France, we do not have national policy regarding ABO compatibility. However, plasma incompatibility is ignored unless the product has hemolysins. In this case, the computer system blocks the delivery to a patient whose ABO group is incompatible with the hemolysins of the product. Regarding cell compatibility, about 84% of the platelets are transfused in a situation of cell compatibility. Cell incompatibility is avoided when the patient is known to produce immune anti-A or -B.

Question 3

- For non-identical platelets, only those without hemolysins are allowed (computer blocking).
- We do not reduce the volume before transfusion in case of ABO incompatibility. In fact, it does not really happen that we cannot apply ABO compatibility for platelets, because we have a resource at the region level that allow to find the right product for the right patient. It is a very rare situation, but this may be the case if the only HLA compatible platelet have hemolysins
- Yes, platelets are stored in PAS.
- We test the donor for anti-A/-B.
- The plasma of the donor is evaluated with a direct agglutination method (microplate technique on OLYMPUS automate). When a donor as a titre > 64, the derived product is labelled. For example O PC with anti-A hemolysins, PC are identified as ‘to be reserved exclusively for a group O or B recipient’, those with anti-A and -B are identified as ‘to be reserved for a group O recipient’.
  a. Yes, it is tested at every donation.
  b. No, the product is not labelled with the titer
  c. All donations are tested for anti A/B, but the result is not used for red blood cell concentrate as the residual volume of plasma is low.
  d. No, we do not transfuse Plasma O to A or B patient, even without antiA/B hemolysins in the plasma.

Question 4

This reaction has been reported for 8 years now, but is very rare. Positive DAT can be observed.

Question 5

There is no policy for RH compatibility of platelets in France. However, when possible, we try to take it into account RHD, especially in women up to the age of 50.

Question 6

We deliver anti-D prophylaxis in women up to the age of 50, when they are not immunosuppressed.

Question 7

- The RBC contamination of platelets is assessed by the visual appearance of the product
- When delivered, the products with a visual appearance of RBC contamination are preferentially delivered to patients who do not rely on RH and KEL compatible protocols

Question 8

Yes.

Question 9

The screening test is recommended between 1 and 4 months after the transfusion. Unfortunately, it is not always realized. However, for the polytransfused patients, in the same region (The French territory is divided in 13 regions), the unique information system allows to detect those cases of immunization, as the screening test is performed before each transfusion. These cases remained rare, and are collected in the haemovigilance system. The rate of anti-D immunization following incompatible transfusion is about 1%.

France Pirenne
Etablissement Français du Sang, Créteil, France
Email: france.pirenne@efs.sante.fr

Michel Raba
Etablissement Français du Sang, Lyon, France
Email: michel.raba@efs.sante.fr

Anne Francois
Etablissement Français du Sang, Paris, France
Email: anne.francois@efs.sante.fr

AUSTRALIA

James Daly & Tanya Powley

Question 1

- Lifeblood provides both Apheresis platelets in PAS and Pooled whole blood-derived platelets in PAS (pool of four donations). SSP+ is the additive solution used for both. All platelets are irradiated and considered leucodepleted. Centrifugation and a Semi Automated Macopress is used for separating whole blood donations but the pooling is a manual process.
- Nationally across the year approximately 36% of platelets are apheresis platelets and the remainder are pooled platelets. However, this split varies between jurisdiction based on clinical and cost considerations.
- Both apheresis and pooled platelets are suspended in SSP+
- Apheresis platelets for paediatrics/neonates are made from splitting a standard apheresis donation, so they have reduced volume and platelet content but otherwise the same specifications

Question 2

There are National guidelines from the ANZSBT and advice from Lifeblood:

- Guidelines for Transfusion and Immunohaematology Laboratory Practice (anzsbt.org.au)
- https://transfusion.com.au/blood_basics/compatibility

These guidelines recommend using ABO identical platelets where possible. However they do acknowledge that this is not always possible and the preferential group selection generally prioritizes the compatibility of the residual plasma in the component and avoidance of minor incompatibility (with the acceptance that group AB platelets are rarely available, and the recommendations that group A2 platelets could be selected for group O or B recipients). They advise that low-titre anti-A/-B apheresis platelets or pooled platelets should be selected for ABO minor incompatible transfusions.

A small unpublished audit done in Queensland for 1 month in 2011 showed that 81% of platelet transfusions were ABO identical. This may not represent current practice.

Question 3

- Only ABO identical transfusions are allowed
- ABO identical platelet transfusions are recommended but due to inventory issues this is not always possible. Few group B and very few group AB platelets are collected, and some hospitals with limited platelet inventory are many hours from our distribution centres so crossing ABO groups for platelet transfusions commonly occurs by necessity.
- Apheresis platelets and pooled platelets are both plasma reduced and suspended in SSP+.
- Apheresis platelet volume specification is 100–400 ml with mean 209 ml. The residual plasma content is approximately 40%
- Pooled platelets volume specification is >160 ml with mean 367 ml. The residual plasma content is approximately 30%
- Yes both apheresis and pooled whole blood-derived platelets are plasma reduced and suspended in PAS (SSP+)

a. Each donation for Clinical plasma or platelets is tested for anti-A/-B. The testing is performed on the donor sample.
b. The anti-A/-B is automated on the PK7300 analyser using the PK7300 plasma dilution ratio 32 and 64, detecting IgM. Validation suggests that the plasma dilution ration 32 in this test system approximates to 1:128 using conventional tube method. If positive for anti-A and/or -B at dilution ratio 32 it is not labelled low titre anti-A/-B. Approximately 60% of donations are negative and labelled as low-titre anti-A/-B. If positive at dilution ratio 64, and the donation is an apheresis platelet donation, manual testing at a titre of 1:8000 is performed and if positive the donation is recalled and the donor deferred – this is to exclude the risk of extremely high titre anti-A/-B apheresis platelet donations being used in incompatible transfusions. This is limited to this donation type because pooled platelets will be lower risk due to dilution with other donations, and clinical plasma is unlikely to be used for incompatible transfusion, and when necessary (e.g., group A plasma instead of group AB for emergency transfusion) low-titre anti-A/-B units are preferentially selected.
c. The donor is tested each time they make a donation for clinical plasma or platelets
d. The titre is not included on the label but the modifier 'low titre anti-A/-B' is printed on relevant labels.
   The apheresis platelets and clinical plasma are labelled as low-titre anti-A/-B if they have tested negative with the PK7300 plasma dilution ratio 32 (and dilution ratio 64). Pooled platelets are labelled low-titre anti-A/-B if all four donors used in the pool tested negative at PK plasma dilution ratio 32. Group AB products are not labelled and red cells are not labelled with 'low anti-A/-B'
e. We do not issue whole blood for transfusion. But yes, we test all donors of donations for clinical components – so all plasma donations are tested.
f. National policies from ANZSBT and advice from Lifeblood for group compatibility for selection of plasma products are available. They are similar but not identical to platelet compatibility, with a stronger avoidance of minor ABO incompatibility for a unit of FFP and no requirement for RhD matching. Cryoprecipitate tends to be grouped with FFP for these policies.

Question 4

Previously anti-A/-B titre was manually performed only on donors making group O apheresis platelet donations. At this time apheresis platelets were suspended in plasma. A very severe case of intravascular haemolysis due to high titre anti-B in a group A donor reported through National Haemovigilance systems, led to the adoption of automated anti-A/B testing for all clinical donations [1]. This testing has continued despite the subsequent move to suspending apheresis platelets in PAS. It would be difficult to determine how common this type of event is from current National Haemovigilance data, However, we are not aware of any subsequent haemolytic reactions due to anti-A/-B in platelet components since the introduction of automated anti-A/-B testing.

Question 5

Yes - There are National guidelines from the ANZSBT and advice from Lifeblood that recommend that particularly RhD-negative females of childbearing potential should receive RhD-negative platelets where possible, and if not possible they should be offered RhD-Ig propylaxis.

- Guidelines for Transfusion and Immunohaematology Laboratory Practice (anzsbt.org.au)
  - 3.3.6 RhD negative patients, especially females of childbearing potential (including female children), should receive RhD negative platelets wherever possible.
  - 3.3.7 If an RhD negative patient receives RhD positive platelets, RhD-Ig should be offered in accordance with institutional policy; this will be at the discretion of the patient's clinician and will depend on the patient's gender, age and diagnosis.
  - 3.3.8 It is not normally necessary to offer RhD-Ig to RhD negative males, postmenopausal women or those (male or female) who are heavily immunosuppressed (e.g., due to haematological malignancy).
  - 3.3.9 If a thrombocytopenic patient requires RhD-Ig, an intravenous (IV) preparation should be considered.

Question 6

Yes anti-D prophylaxis is advised for RhD-negative females of childbearing potential that receive RhD-positive platelet transfusions.

Question 7

We do not routinely test platelets for red cell contamination. Visual inspection against a reference guide is performed with an acceptable limit equivalent to 1.0 ml red cells per pack.

Question 8

Yes – these policies and advice apply to all types of platelets.

Question 9

There is no consistent mechanism to detect and record cases of alloimmunization to RhD due to platelet transfusion … or any other cause at a National level.

One jurisdictional Haemovigilance system – the Serious Transfusion Incident Reporting (STIR) System, which covers several Australian

States and Territories captures incidents related to RhD-Ig request or administration including following mismatched red cell or platelet transfusion. STIR also captures cases of delayed serological transfusion reaction (alloimmunization) if detected 24 h to 3 months after transfusion.

James Daly
Australian Red Cross Lifeblood, Brisbane, QLD, Australia
Email: jamedaly@redcrossblood.org.au

Tanya Powley
Australian Red Cross Lifeblood, Brisbane, QLD, Australia
Email: tpowley@redcrossblood.org.au

REFERENCE

1. Swain F, Daly J, Baidya S, Wilson B, Morrison J, Liew YW, Powley T, Jivan Y, Bryant S, Allen A, Crampton N. Acute haemolytic reaction secondary to an ABO minor mismatched platelet transfusion from a group A blood donor. Transfus Med. 2019;29:133-135.

ORCID

Rounak Dubey https://orcid.org/0000-0002-9467-5759
Magnus Jöud https://orcid.org/0000-0002-4734-0040
Joan Cid https://orcid.org/0000-0001-5445-4508
Miquel Lozano https://orcid.org/0000-0003-2593-833X
Peter Flanagan https://orcid.org/0000-0001-5270-3910
Gwen Clarke https://orcid.org/0000-0001-7738-3378
Salwa Hindawi https://orcid.org/0000-0003-3804-5434
Jose Mauro Kutner https://orcid.org/0000-0003-3784-6731

How to cite this article: Cardigan R, New HV, Estcourt L, Zhiburt E, Dubey R, Bengtsson J, et al. International Forum on Policies and Practice for Transfusion of ABO and RhD Non-Identical Platelets: Responses. Vox Sang. 2021;1–20.

Received: 17 March 2021 | Accepted: 1 April 2021

DOI: 10.1111/vox.13124

LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

For whole blood research, look to the whole world

We read with interest the recent review from the Biomedical Excellence for Safer Transfusion (BEST) collaborative, which described the history, storage, and safety of whole blood for traumatic haemorrhage. [1] As the authors note, there has been a renaissance in whole blood research in high-income nations, due in large part to promising results from military studies. While we agree with the authors' assertion that this is a true global health issue and applaud the international representation within the BEST collaborative, we were surprised that sub-Saharan African data were not included in this review. Many countries in sub-Saharan Africa have utilized whole blood transfusion for haemorrhaging patients for decades. Although this was initially and primarily driven by the inability to implement blood component separation techniques, many countries have since developed this technology but still utilize whole blood. In Malawi, for example, where component separation techniques were introduced in 2005, whole blood is still used to treat haemorrhage.

Large prospective studies of whole blood utilization and safety could be conducted in the sub-Saharan African region, which leads the world in population growth. Research investments may mitigate blood supply shortages and although trial-level data from sub-Saharan Africa remains limited, there are descriptive studies on the use of whole blood from this region to lay the groundwork for clinical trials. These studies predominantly describe transfusion practices for major obstetric haemorrhage. Although controversy persists regarding the use of whole blood for women of childbearing age, recent case reports showcase the safe use of low-titre O-positive whole blood (LTO + WB) for postpartum haemorrhage. [2, 3] In a 2019 study in Nigeria, the authors showed that 58% of all whole blood requisitions (947/1,636) were for obstetric patients. [4] A 2013 nationally representative cross-sectional study of 42 Tanzanian hospitals revealed that 92% ($n = 219{,}346$; SD 4,150) of transfusion requests were for whole blood and 21% were for obstetric haemorrhage [5].

Investigators have not yet seized the opportunity to collaborate on whole blood transfusion research in the sub-Saharan African region. Even the WOMAN trial, a large and rigorous international trial that enrolled 62% of its participants from Africa, was critiqued for lack of data describing blood transfusion practices. Currently, the major obstacle to clinical research in the sub-Saharan African region is the limited health and research infrastructure, which makes the acquisition of granular clinical data difficult. Nevertheless, the long history and high volume of whole blood transfusion in this region makes clear its potential to lead the world in whole blood research.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

FUNDING INFORMATION

The authors report no funding for this manuscript.

Meghan Prin[1]
Thomas Mfune[2]
Stephen Njolomole[3]
Ernest Eugene Moore[4]
Adit Ginde[5]

[1]*Department of Anesthesiology, University of Colorado, Aurora, Colorado, USA*
[2]*Malawi Blood Transfusion Services, Lilongwe, Malawi*
[3]*University of Malawi College of Medicine, Department of Pathology, Blantyre, Malawi*
[4]*Department of Surgery, Ernest E Moore Shock and Trauma Center at Denver Health, Denver, Colorado, USA*
[5]*Department of Emergency Medicine, University of Colorado, Aurora, Colorado, USA*

Correspondence
Meghan Prin, 12401 East 17th Avenue, Leprino Building, 7th Floor, Aurora, CO 80045, USA.
Email: meghan.prin@cuanschutz.edu

ORCID

Meghan Prin https://orcid.org/0000-0002-2886-4666

REFERENCES

1. Hervig TA, Doughty HA, Cardigan RA, Apelseth TO, Hess JR, Noorman F, et al. Re-introducing whole blood for transfusion: considerations for blood providers. Vox Sang. 2021;116:167–74.
2. Bahr TM, DuPont TL, Morris DS, Pierson SE, Esplin MS, Brown SM, et al. First report of using low-titer cold-stored type O whole blood in massive postpartum hemorrhage. Transfusion. 2019;59:3089–92.
3. Morris DS, Braverman MA, Corean J, Myers JC, Xenakis E, Ireland K, et al. Whole blood for postpartum hemorrhage: early experience at two institutions. Transfusion. 2020;60:S31–5.
4. Okoroiwu HU, Okafor IM. Demographic characteristics of blood and blood components transfusion recipients and pattern of blood utilization in a tertiary health institution in southern Nigeria. BMC Hematol. 2018;18:16.
5. Apata IW, Drammeh B, De AK, Bjork A, Pathak S, Lyimo M, et al. Diagnoses and ordering practices driving blood demand for treatment of anemia in Tanzania. Transfusion. 2018;58:379–89.

Received: 19 May 2021 | Accepted: 19 May 2021

DOI: 10.1111/vox.13163

LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Reply to Watchko and Maisels: Exchange transfusion in Rh haemolytic disease

We thank Dr. Watchko and Dr. Maisels for their knowledgeable comments on our study and would like to address their questions.

One concern was the use of a higher bilirubin threshold for phototherapy and exchange transfusion (ET) at our centre for infants with haemolytic disease of the foetus and newborn (HDFN). The relationship between haemolysis and high bilirubin levels is clear, but it is unclear whether infants with HDFN have a higher risk of bilirubin neurotoxicity compared to infants without HDFN in case of similar levels of serum bilirubin [1]. We have no reason to assume that HDFN alters the blood–brain barrier and therefore do not assume that the studied, term infants have a higher risk of bilirubin encephalopathy. Moreover, the maximum bilirubin level at birth remained stable for group II (2005–2015) and III (2015–2020), with maximum values of 257 (standard deviation [SD] of 89) and 262 μmol/L (SD of 80) after birth. Local policy also indicates the near-immediate start (within 15 min after birth) of intensive phototherapy for infants with HDFN regardless of the first measured bilirubin after birth or other risk factors and will therefore not delay treatment. Other risk factors include prematurity, asphyxia, suspected infection/sepsis and low albumin levels.

With regard to phototherapy, technological advancements of the used (LED) lamps very likely also contributed to more effective treatment of hyperbilirubinaemia and decreased use of ET. In our study, the median duration of phototherapy per infant remained stable over the years, with a median of 4 (interquartile range [IQR] 3–5), 5 (IQR 3–6) and 5 (IQR 4–6) days in our three time cohorts. The timing of the start of phototherapy (within 15 min after birth) has not changed over the years.

The relationship between intrauterine transfusion (IUT) and ET(s) was previously reported by our study group [2, 3]. Infants treated with more IUTs required fewer ET(s); the rate of ET dropped from 39% of infants treated with one IUT to 25% after two IUTs and further declined to 8% for infants treated with five IUTs (infants born 2005–2018) [2]. The decline in ET rate in this study does not show a similar trend as the IUT rate has declined in the three time cohorts in our study from a median of three IUTs (IQR 2–4) in group I to two (IQR 2–4) and two (IQR 1–3) in group II and III.

The standard blood product in the Netherlands for neonatal ET has a haematocrit of around 0.50; no additional albumin is transfused prior to or during the procedure. As stated, the product consists of a two donor combination of washed red blood cells and adult plasma.

We hope to have clarified the raised issues and welcome all further thoughts on our study.

ACKNOWLEDGEMENTS

The authors received no specific funding for this work.

CONFLICT OF INTEREST

There are no conflicts of interest to report.

Isabelle M. C. Ree[1,2]
Carolin F. J. Besuden[1]
Vivianne E. H. J. Wintjens[1]
Joanne (E.) J. T. Verweij[3]
Dick Oepkes[3]
Masja de Haas[2,4,5]
Enrico Lopriore[1]

[1]*Department of Paediatrics, Division of Neonatology, Leiden University Medical Centre, Leiden, The Netherlands*
[2]*Centre for Clinical Transfusion Research, Sanquin, Leiden, The Netherlands*
[3]*Department of Obstetrics, Division of Foetal Medicine, Leiden University Medical Centre, Leiden, The Netherlands*
[4]*Department of Immunohaematology and Blood Transfusion, Leiden University Medical Centre, Leiden, The Netherlands*
[5]*Department of Immunohaematology Diagnostics, Sanquin, Leiden, The Netherlands*

Correspondence
Isabelle Ree, Department of Paediatrics, Leiden University Medical Centre, J6-S, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands.
Email: i.m.c.ree@lumc.nl

AIsabelle M.C. Ree and Carolin F.J. Besuden contributed equally to this study.

ORCID

Isabelle M. C. Ree https://orcid.org/0000-0003-3608-3509

REFERENCES

1. Kaplan M, Bromiker R, Hammerman C. Hyperbilirubinemia, hemolysis, and increased bilirubin neurotoxicity. Semin Perinatol. 2014;38:429–37.
2. Ree IMC, Lopriore E, Zwiers C, Böhringer S, Janssen MWM, Oepkes D, et al. Suppression of compensatory erythropoiesis in hemolytic disease of the fetus and newborn due to intrauterine transfusions. Am J Obstet Gynecol. 2020;223:119.e1–119.e10.
3. Ree IMC, de Haas M, Millderburg RA, Zwiers C, Oepkes D, Bom JG, et al. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the fetus and newborn in the first 3 months after birth. Br J Hematol. 2019;186:565–7.

Received: 30 November 2021 | Accepted: 30 November 2021

DOI: 10.1111/vox.13231

OBITUARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Anneke Brand (1946–2021)

Anneke Brand, Professor in Transfusion Medicine, passed away on Sunday, 21 November 2021, at the age of 75. She was a pioneer in both the immunological and clinical aspects of blood transfusion. She has played a pivotal role in the development of the Dutch Cord Blood Bank. During her entire career, she was dedicated to inspiring many researchers and clinicians alike in the field of transfusion medicine and haematology. Her ability to question and challenge scientific findings, as well as many social issues, is unequalled.

Anneke was born on 7 June 1946 in The Hague, the Netherlands. In 1973, she obtained her medical degree from the Free University Amsterdam and was accepted for a PhD project at the Department of Immunohaematology and Blood Bank of the Academic Hospital Leiden under the wings of Prof. Jon van Rood and Dr George Eernisse. In this project, she started working on the management of patients refractory to platelet transfusions, including antibody detection and donor selection. In those days, many leukaemia patients suffered from severe bleeding complications and presented with unexplained severe transfusion reactions and markedly reduced post-transfusion increments. In the laboratory of the Immunohaematology Department, important aspects of the human leukocyte antigen (HLA) class type I system were unravelled and the first sibling bone marrow transplants for severe combined immunodeficiency and aplastic anaemia patients were performed. Laboratory tests to detect HLA antibodies were developed. In 1978 she defended her thesis 'Platelet Supportive Care', and meanwhile learned all aspects of blood-banking. After this, she started her training in internal medicine in Haarlem, followed by her specialization in haematology at the Leiden Academic Hospital. As a medical specialist, in 1984 she returned to the Blood Bank and Haemapheresis Department, combining patient care and clinical research, investigating the role of leukocytes on clinical transfusion outcomes in various disorders, from colorectal cancer surgery to erythrocyte alloimmunization. Her research led to the adoption of leukoreduction to diminish alloimmunization as well as other adverse transfusion reactions. After a sabbatical in Paris in the laboratory of Prof. Michel Kazatchkine working on anti-idiotypic antibodies in intravenous immunoglobulins, in 1988 she succeeded George Eernisse as Medical Director of the Red Cross Leiden and the Blood Bank of the Leiden University Hospital. After the AIDS drama, good manufacturing practice became mandatory and regional Dutch blood banks merged with neighbouring blood banks. Over a period of 10 years, all Dutch Red Cross blood banks gradually merged with the Dutch Laboratory of the Red Cross to form one national organization: Sanquin. In 1994, together with Fred Falkenburg, she established the Dutch Cord Blood Bank, a joint effort between the Leiden University Medical Centre and the Red Cross Blood Bank. In 1995, she joined the Royal College of Pathology, and in 1999 she became Professor of Internal Medicine with assignment to Transfusion Medicine. In that period, she started researching the optimal conditions for the storage and expansion of cord blood for transplantation as well as exploring the possibility of using cord blood to produce red blood cells for transfusion. From 2000 till her 'age-enforced' retirement in 2011, she was the head of clinical research at Sanquin Blood Bank Southwest. In this period, several clinical trials were successfully conducted, including studies regarding peri-operative blood management strategies as well as several clinical platelet transfusion trials. After her retirement, she remained active in many fields, including the board of the European Haematology Association (2012–2016), Europdonor (till 2015), and advisory work for TRIP, the Dutch Haemovigilance network. She received the Claes Högman Lecture Award of the Swedish Blood Transfusion Society (1997), the Blundell Award of the British Blood Transfusion Society (2009), the Presidential Award of the International Society of Blood Transfusion (2020), and, very recently, the Landsteiner Award (2021). Scientifically,

she continued working on non-inherited maternal antigens, an interest she shared with Jon van Rood, and studying factors that influence erythrocyte alloimmunization.

Only recently were we informed that Anneke was severely and incurably ill, and her passing away was abrupt. We will remember her words of comfort, wisdom, and inspiration, as we will remember her tremendous scientific drive and encyclopaedic up-to-date knowledge in many fields of medicine. We will never forget the detailed questions she used to have at the end of every lecture she attended, aiming to improve the quality of everyone's contribution to transfusion medicine. She will be deeply missed by her family, friends, and colleagues.

Jean-Louis H. Kerkhoffs[1,2]
Cynthia So-Osman[2,3]

[1]*Department of Haematology, Haga Teaching Hospital, The Hague, The Netherlands*
[2]*Department of Unit Transfusion Medicine, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands*
[3]*Department of Haematology, Erasmus Medical Center, Rotterdam, The Netherlands*

Correspondence
Jean-Louis H. Kerkhoffs, HagaZiekenhuis, Els Borst-Eilersplein 275, 2545 AA Den Haag, The Netherlands.
Email: j.kerkhoffs@hagaziekenhuis.nl

ORCID
Cynthia So-Osman https://orcid.org/0000-0003-4151-2865

DOI: 10.1111/vox.13136

DIARY OF EVENTS

See also http://www.isbtweb.org/congresses/

10.2.2022	The European Hematology Association (EHA) and the European Society for Blood and Marrow Transplantation (EBMT) - 4th edition of the jointly organized European CAR T-cell Meeting.
15-16.3.2022	The IPFA/EBA Symposium on Plasma Collection and Supply will take place fully physical in Amsterdam, the Netherlands on March 15 - 16, 2022.
23.3.2022	Eye Drops from Human Origin - First EDHO Workshop on Current Standards and Future Developments organized by the ISBT Working Party Cellular Therapies.

