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Vox Sanguinis

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IN THIS ISSUE

Journals and affiliated medical societies must address gender inequities among editors
White paper on pandemic preparedness in the blood supply
Impact of donor ferritin testing on iron deficiency prevention and blood availability in France: A cohort simulation study
Efficacy of therapeutic plasma exchange in severe COVID-19 disease: A meta-analysis

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Vox Sanguinis

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Vox Sanguinis

International Journal of Blood Transfusion

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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 eight main sections:

1. Donors and Donations: Donor recruitment and retention; Donor selection; Donor health (vigilance, side effects of donation); Big data analysis and blood donation.
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3. 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.
4. Transfusion Medicine and New Therapies: Transfusion practice, thresholds and audits; Transfusion efficacy assessment, clinical trials; Non-infectious transfusion adverse events; Therapeutic apheresis.
5. Haemovigilance: Near misses, adverse events and side effects throughout the transfusion chain; Monitoring, reporting and analysis of those adverse events and side effects; Activities aiming at increasing the safety of the whole transfusion chain; Standardization of the definition of adverse events and side effects.
6. Patient Blood Management: Caring for patients who might need a transfusion; Transfusion indication decision-making process; Search for the optimal patient outcomes; Study of transfusion alternatives; Autologous blood transfusion.
7. Immunohaematology: Red cell, platelet and granulocyte immunohaematology; Blood phenotyping and genotyping; Molecular genetics of blood groups; Alloimmunity of blood; Pre-transfusion testing; Autoimmunity in transfusion medicine; Blood typing reagents and technology; Immunogenetics of blood cells and serum proteins: polymorphisms and function; Complement in immunohaematology; Parentage testing and forensic immunohaematology.
8. Cellular Therapies: Cellular therapy (sources; products; processing and storage; donors); Cell-based therapies; Genetically modified cell therapies; Stem cells (sources, collection, processing, storage, infusion); Cellular immunotherapy (e.g., CAR-T cells, NK cells, MSC); Cell-based regenerative medicine; Molecular therapy; In vitro manufacturing of blood components.

Vox Sanguinis also publishes the abstracts associated with international and regional congresses of the ISBT. (Abstracts from meetings other than those held by the ISBT are not accepted.)

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Vox Sanguinis (2024) **119**, 405–515

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119/5/2024

Contents

Original Articles

Donors and Donations

409 Advancing gender inclusivity for Two-Spirit, trans, nonbinary and other gender-diverse blood and plasma donors J. Haw, T. Butler-Foster, B. Murray, D. Lapierre, J. Bosse, J. Edwards, Ş. Gümüşpala, C. Jenkins & A. Devor

417 Red cell concentrates from teen male donors contain poor-quality biologically older cells O. Mykhailova, M. Brandon-Coatham, C. Phan, M. Yazdanbakhsh, C. Olafson, Q.-L. Yi, T. Kanias & J. P. Acker

428 Voluntary blood donation preferences in China: A discrete choice experiment among experienced and inexperienced donors C. Cao, J. Wang, T. Guo, X. Hu, B. Li, C. Wu, Z. Liu & S. W. Pan

Blood Component Collection and Production

439 A tight interplay between platelet activation and mitochondrial DNA release promotes platelet storage lesion in platelet concentrates K. Haeri, S. Samiee, P. Beigi, S. Hajati & M. R. Deyhim

Transfusion-transmitted Disease and its Prevention

447 Blood donation screening for hepatitis B virus core antibodies: The importance of confirmatory testing and initial implication for rare blood donor groups M. X. Fu, J. Ingram, C. Roberts, V. Nurmi, E. Watkins, N. Dempsey, T. Golubchik, J. Breuer, S. Brailsford, W. L. Irving, M. Andersson, P. Simmonds & H. Harvala

Transfusion Medicine and New Therapies

460 Blood lactate after pre-hospital blood transfusion for major trauma by helicopter emergency medical services B. Mitra, C. S. Talarico, A. Olaussen, D. Anderson & B. Meadley

467 Evaluation of the impact of diagnostic blood loss and red blood cell transfusion in very-low-birth-weight anaemic neonates during hospitalization: A multi-centre retrospective clinical study T. Ma, J. Yang, Y. Sun, A. Song, J. Zhang, Y. Shen, K. Hua, W. Wu & W. Chen

476 Trends in category and grade for therapeutic plasma exchange in the latest guideline on therapeutic apheresis by the American Society for Apheresis: Hurdles in pursuing evidence-based medicine H. J. Kim, Y. Chung, H. Kim, S.-H. Hwang, H.-B. Oh & D.-H. Ko

483 Haemolytic transfusion reactions caused by non-ABO red cell antibodies reported to the Norwegian Haemovigilance System 2004–2020 A. Espinosa, C. T. Steinsvåg & Ø. Flesland

490 Gender differences in scholarly productivity of early-career transfusion medicine physicians T. S. Ipe, Y. C. Tanhehco, G. S. Booth & B. D. Adkins

Immunohaematology

496 Evidence that CD36 is expressed on red blood cells and constitutes a novel blood group system of clinical importance A. G. Alattar, J. R. Storry & M. L. Olsson

Report

505 The future of blood services amid a tight balance between the supply and demand of blood products: Perspectives from the ISBT Young Professional Council A. Lewin, E. McGowan, J. Ou-Yang, L. A. Boateng, C. L. Dinardo, S. Mandal, N. Almozain, J. Ribeiro & S. L. Sasongko, on behalf of the Young Professional Council of the ISBT

Obituary

514 Dirk de Korte (1955–2024) P. F. van der Meer, J. Lagerberg & T. R. L. Klei

515 Events

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ORIGINAL ARTICLE

Advancing gender inclusivity for Two-Spirit, trans, nonbinary and other gender-diverse blood and plasma donors

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Abstract

Background and Objectives: Two-Spirit, trans, nonbinary and other gender-diverse (2STGD) donors face challenges in donation. While many blood operators aim to address these challenges, to date, no empirical study with these donors has been conducted to guide their efforts. This paper reports 2STGD donors' views on a two-step approach asking donors their gender and sex assigned at birth (SAAB), and expanding gender options in donor registration.

Materials and Methods: A qualitative community-based study was conducted with 2STGD donors ($n = 85$) in Canada. Semi-structured, in-depth interviews were conducted from July to October 2022, audio-recorded and transcribed. Data were analysed using a thematic analytic framework.

Results: Participants were divided on their views of a two-step approach asking gender and SAAB. Themes underlying views in favour of this approach included the following: demonstrating validation and visibility, and treating 2STGD donors and cisgender donors alike. Themes underlying views not in favour or uncertain included potential for harm, compromising physical safety, and invalidation. All participants were in favour of expanding gender options if blood operators must know donors' gender.

Conclusion: Results indicate that a two-step approach for all donors is not recommended unless the blood operator must know both a donor's gender and SAAB to

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ensure donor and/or recipient safety. Gender options should be expanded beyond binary options. Ongoing research and evidence synthesis are needed to determine how best to apply donor safety measures to nonbinary donors.

Keywords
blood donors, donor screening, gender diversity, nonbinary, plasma donors, qualitative methods, transgender

Highlights

- Results indicate that a two-step approach (i.e., asking donors their gender and sex assigned at birth [SAAB]) for all donors is not recommended unless it is imperative that the blood operator know both a donor's gender and SAAB to ensure donor and/or recipient safety.
- Results indicate that gender options should be expanded beyond binary options of woman/female and man/male if blood operators must know a donor's gender.
- Additional research and evidence synthesis should be conducted to determine how best to apply donor safety measures for nonbinary donors.

INTRODUCTION

Two-Spirit, trans, nonbinary, and other gender-diverse blood and plasma donors (henceforth 2STGD donors) face challenges in donation and have increasingly advocated for more inclusive and affirming donation policies and practices [1] (see Supporting Information S1 for glossary of terms). For example, a significant barrier for some 2STGD donors is a lack of gender/sex options beyond woman/female (W/F) or man/male (M/M). While many blood operators recognize the importance of ensuring that the donation experience is affirming and inclusive for 2STGD donors, to date, no empirical study with these donors has been conducted to guide their efforts [2, 3].

Blood operators use a donor's gender or sex/sex assigned at birth (SAAB) to ensure recipient and donor safety [3]. First, for blood operators using gender-specific donor questionnaires, different pregnancy questions and/or different versions of the men-who-have-sex-with-men (MSM) questions may be asked (to minimize the risk of transfusion-related acute lung injury and transfusion-transmitted infection). Second, a donor's gender/SAAB is used to determine donor safety criteria including estimated blood volume (EBV), minimum haemoglobin level, inter-donation period and ferritin cut-off values. Third, gender/SAAB is used to direct component production and testing such as programming apheresis collection and using plasma from donors registered as W/F for fractionation and not transfusion [3]. Any changes to how a donor's gender/SAAB is registered must consider both impacts on 2STGD donors, and how gender/SAAB is used for screening and processing.

Canadian Blood Services (CBS), one of two blood operators in Canada, is responsible for the collection and distribution of blood and blood products to all provinces and territories except Quebec. Any changes to screening criteria that may impact recipient safety must be approved by Health Canada, the regulator, before implementation. Beginning in 2016, a standardized approach was implemented to screen 2STGD donors. Donors who self-disclosed as 2STGD were registered in their declared gender if they had had genital gender-affirming surgery, or in their SAAB if they had not [3]. With the implementation of sexual-behaviour-based screening (SBBS) for all donors in September 2022, 2STGD donors are no longer asked if they have had genital gender-affirming surgery. SBBS refers to replacing the gendered MSM questions with gender-neutral sexual behaviour-based questions asked to all donors. With this change, the donor's gender/SAAB is no longer needed to guide which version of the questions they are asked. Currently, all binary donors are registered in their self-declared gender. While this is a significant improvement, nonbinary donors must still register in a binary gender or SAAB.

Globally, blood operators have different practices for registering donors' gender/SAAB [4]. This range is due, in part, to laws and policies that impact 2STGD people. These include laws in some countries that limit or prohibit 2STGD people's ability to live visible and authentic lives in their felt gender, and the lack of laws and policies to ensure physical and psychological safety for them. Where there is some consistency, however, is that most blood operators [4, 5] offer only binary gender/sex options (i.e., W/F or M/M) to donors since most rely on blood establishment computer systems (BECS) and automated collection devices that only allow for two gender/sex options. Data from one blood operator in the US that recently added gender options (i.e., F, M, Trans and Other) show no compromise to safety [5].

Cisnormativity underlies the erasure of 2STGD people [6]. Cisnormativity is a system of thought that includes the following assumptions: a person's gender is determined by, and always aligns with, SAAB; gender does not change over time, and everyone's gender and SAAB is the same and binary (woman = female or man = male). Any person whose gender and/or sex is not consistent with these assumptions is considered an outlier with attendant social, cultural, legal, health, and political consequences [6, 7]. Whether consciously or not, cisnormative assumptions that can cause harm to 2STGD donors are built into donor systems, processes, and practices.

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Providing more inclusive gender options is not a unique consideration only for blood operators. Research on survey design for health and population-level data recommends a two-step approach (asking both gender and SAAB), which is more gender inclusive and accurate than a single question [8, 9]. A two-step question can address erasure of 2STGD people in research and bureaucratic systems [6], provided that options for gender and SAAB are not limited to binary options. Addressing erasure of 2STGD people in health and population survey data is a necessary first step to ensuring that they receive adequate healthcare resources and services. For healthcare settings that provide clinical care for 2STGD people, additional considerations may be necessary [10].

What remains unclear is whether the recommendation of a two-step question in surveys is transferable to blood donation. Suggested options that may improve the current gender limitations include the following: (1) moving to a two-step question for all donors; (2) keeping a single question asking gender and expanding gender options; and/or (3) moving to a more gender-neutral donation process overall whereby knowing a donor's gender is not necessary. These options are not mutually exclusive and given the significant impact on 2STGD donors, it is imperative to understand their views on these different options. Moreover, if additional gender options are provided to donors, the views of 2STGD people must be prioritized when determining which options to include since they will be most affected by options offered.

This paper reports results from a qualitative community-based study that was initiated in 2022 before Health Canada approval, and CBS' implementation of, SBBS and changes for 2STGD donors. Since 2STGD people and communities have not always been well-served by research and researchers [11, 12], we applied a community-based methodological approach that foregrounds co-learning between researchers and community members, shared decision-making, and benefits to communities involved [13–15]. We conducted an exploratory study to (1) better understand 2STGD donors' experiences of the donation process; (2) identify barriers and enablers to an inclusive, affirming, respectful and safer donation experience for 2STGD donors; and (3) explore 2STGD donors' views on the donor questionnaire and expanding gender options. Results presented below focus on the third objective and report participants' views on a two-step question and expanding gender options for donors. In the discussion, we apply study results to various options to advance gender inclusivity for 2STGD donors.

MATERIALS AND METHODS

This project applied ethical guidelines for research with 2STGD communities as outlined by the Canadian Professional Association for Transgender Health [16]. A Community Advisory Group of 2STGD community members provided feedback on study materials and guidance throughout the research process. REB approval was obtained from CBS (Ottawa, Canada) and the University of Victoria (Victoria, Canada).

Participant recruitment and data collection

Participants were recruited through the CBS donor database. Eligibility criteria included (1) self-identify as 2STGD, (2) 17 years and older, (3) donated or tried to donate blood and/or plasma in the previous 24 months and (4) comfortable in English. Because not all 2STGD donors would have self-identified when they donated, recruitment included two groups of donors: (1) donors known by the blood operator to be 2STGD and (2) general donor pool. All donors in group 1 who met eligibility criteria were sent a recruitment email ($N = 360$). Three thousand donors in group 2 were randomly selected to receive a recruitment email. Initially, we aimed to recruit 30–40 participants; however, given the high level of interest and our commitment to hearing from everyone who wanted to participate, everyone who expressed interest and met eligibility criteria was included for a total of 85 participants. Because we cannot determine whether a participant was recruited through group 1 or 2, we cannot calculate a response rate for each group.

Data were generated through in-depth, semi-structured interviews conducted July–October 2022. Participants had the option to be interviewed by telephone or videoconference, and to be interviewed by a transgender, nonbinary, or cisgender member of the study team. Interview topics included experiences with blood/plasma donation, experiences completing the donor questionnaire, and views on expanding gender options in donor screening. Open-ended questions enabled participants to share what was relevant to them to ensure that data were grounded in the experiences and views of the participants. Interviews were 20–75 min long, audio-recorded with participants' consent and transcribed. Following transcription, participants were able to review their transcript. Participants were offered a $25 e-gift card.

Data analysis

Data analysis was guided by an interpretivist epistemological approach. According to this view, phenomena are known primarily through the meanings, interpretations, and ideas that people use to make sense of, or construct, the phenomena of interest [17]. Because a guiding research question was to understand 2STGD donors' views on potential improvements to registering donors' gender, we used thematic analysis for its methodological flexibility [18, 19]. First, deductive coding of participants' views as either in favour of changing to a two-step question, uncertain about this change, or not in favour of this change was completed. Second, open and inductive interpretive coding was completed to understand the reasons, assumptions and meanings underlying their views [18, 19].

Data were coded by three members of the research team. Each person independently read an interview transcript making note of both categorical and a priori codes (e.g., views on a two-step question), and interpretive or thematic codes (e.g., demonstrating validation and visibility). Following this in-depth read, coders met to discuss and develop a codebook. All three then applied the codebook

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TABLE 1 Characteristics of study sample and all known 2STGD donors.

Characteristics	Study participants ($n = 85$) Number (%)	Known 2STGD donors ($N = 450$)[a] Number (%)
Age		
17–19	4 (4.7)	30 (6.7)
20–29	40 (47.1)	239 (53.1)
30–39	26 (30.6)	122 (27.1)
40–49	10 (11.8)	38 (8.4)
50+	5 (5.9)	21 (4.7)
Gender		
Trans woman/transfeminine[b]	23 (27.1)	-
Trans man/transmasculine[c]	41 (48.2)	-
Nonbinary	20 (23.5)	-
Agender	1 (1.2)	-
Gender/SAAB[d]		
Female	-	298 (66.2)
Male	-	152 (33.8)
Ethnicity		
Indigenous/Aboriginal	1 (1.2)	10 (2.2)
Asian/E Asian/SE Asian	4 (4.7)	33 (7.3)
White/Caucasian	70 (82.4)	326 (72.4)
Filipino	2 (2.4)	1 (0.2)
Latin American/Hispanic	1 (1.2)	4 (0.9)
South Asian	1 (1.2)	8 (1.8)
Multi-ethnic	6 (7.1)	15 (3.3)
Arabic/Middle Eastern	-	2 (0.4)
Black/Black Caribbean	-	4 (0.9)
Other	-	30 (6.7)
No response	-	17 (3.8)
Number of donations		
1	6 (1.3)	-
2–3	16 (18.8)	-
4–9	26 (30.6)	-
10–19	23 (27.1)	-
20–29	7 (8.2)	-
30–39	4 (4.7)	-
40+	3 (3.5)	-
Donor status		
New donor (1 donation)	6 (1.3)	190 (42.2)
Return donor (>1 donation)	79 (92.9)	260 (57.8)

[a]Donors with a 6500 code were 0.08% of the total donors over the same period ($N = 562{,}513$). Only 360 of the 450 donors were sent a recruitment email.
[b]Participants provided their gender in free text. Trans woman/transfeminine also includes transgender woman, trans female and female.
[c]Participants provided their gender in free text. Trans man/transmasculine also includes transgender man, trans male and 'just M'.
[d]The gender/SAAB categories provided here are the options provided by Canadian Blood Services.

independently to a second interview transcript and noted any differences in the interpretation of codes to ensure clarity of the codebook and relative consistency across coders. Following this second step, two members coded the remainder of the interview transcripts using *Nvivo 12*. All coders met weekly during the coding process to discuss the process, analytic themes, and any new emergent codes. Disagreements between coders were resolved through discussion and all coders agreed on the final codebook.

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Participant characteristics

More than three-quarters of participants were between the ages of 20 and 39 (77.7%), the largest proportion self-identified as trans man/transmasculine (48.2%), followed by trans woman/transfeminine (27.1%), and nonbinary (23.5%). Most were White (82.4%) and return donors (92.9%) and had donated over four times (74.1%). Compared with all known 2STGD donors who had donated from June 2020 to September 2022 (study recruitment period), slightly more participants were in the 40+ age groups, participants were less ethnically diverse, and more were return donors. Comparing gender characteristics for these two groups is not possible since donors are limited to binary options only in donor registration. It is noteworthy that 25% of participants—those who are nonbinary or agender—could not be registered in their gender within the current binary system (see Table 1).

RESULTS

Participants' views were mixed on a two-step approach asking gender and SAAB with 50.6% ($n = 43$) in favour of this change, 21.2% ($n = 18$) not in favour, and 28.2% ($n = 24$) uncertain about this change. In presenting thematic results below, we have combined participants who were not in favour and uncertain since these two groups expressed similar themes concerning the reasons for, and meanings underlying, their views.

In favour of a two-step approach

Demonstrating validation and visibility

Moving from a single question asking gender or SAAB with binary options only, to a two-step approach of asking gender and SAAB was viewed as validating and giving visibility to gender diversity among donors. Participants whose gender was not offered by the options of F or M provided by CBS felt invisible or erased. This was particularly the case for nonbinary participants and for those 2STGD participants who wanted their gender to be visible. One participant explained that a two-step question signalled that they would be visible in 'people's thought processes and systems' (P09).

Validation and visibility were also related to clarity in the donation process. Some participants found that the current limitations on gender registration led to ambiguity regarding if they should indicate to donor centre staff that they are 2STGD:

> At least there would be an opening for conversation instead of me feeling like, do I just tell them [I am trans], or do I wait 'til they ask? Sometimes that's a little awkward and I'm never sure how to bring it up. (P45)

These participants assumed that blood operators need this information. However, without a clear question asking if they are 2STGD, they found the current process confusing and, at times, inconsistent because of different staff practices. On this point, a few participants wanted to be asked directly if they are transgender if the blood operator needs to know, and then to have this question followed with a two-step question. Several also mentioned that a two-step question was preferred because it is consistent with changes made to the latest version of the Canadian census (2021).

Being treated the same as cisgender donors

During the interview, participants were told that if a two-step question were implemented, it would be asked to all donors. This was viewed by some as an improvement because it meant that they would be treated the same as cisgender donors. Many interpreted this to mean that they would not be singled out and asked additional questions about genital gender-affirming surgery, as they had been prior to the changes in September 2022.

Not in favour or uncertain of a two-step approach

Potential for psychological harm

For many participants not in favour, or uncertain, of a two-step approach, asking SAAB not a neutral question because it evokes past experiences that caused psychological distress. In particular, participants expressed concerns that asking SAAB could cause psychological harm to 2STGD people 'who have endured a lot of trauma around their sex and gender' (P07). Being asked explicitly their SAAB was described as a reminder that their gender does not align with their SAAB. One participant explained that being asked their SAAB is a reminder of 'a body and a person that is not them' (P44), while another explained that they 'would be against a [two-step question] because when a person transitions, their dead life is their dead life' (P81). Participants also spoke about feeling discomfort because they considered asking SAAB to be asking about their genitals and therefore highly 'intrusive' (P84). For these participants, being asked their SAAB would be asking them to verify someone who they are not, with the potential for psychological harm caused by being reduced to their genitals. Others described the potential psychological harm of being judged by staff who may not understand or accept gender diversity.

Having to out themselves and compromise safety

In addition to potential psychological harm, participants expressed concerns with a two-step approach requiring 2STGD donors to out themselves when both questions are asked. Some participants explained that being out as trans 'opens people up to discrimination and transphobia' (P70). That is, providing this information carries with it risks to their safety. A two-step approach also raises the potential to be inadvertently outed if other donors or staff overhear their

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responses, or if staff speak about their responses in a public area, compromising the physical safety of 2STGD donors. Some participants did not feel safe enough to be out in their communities, especially in smaller communities. For some, the potential to be outed, coupled with the risk of having their gender be 'something that can be used against you down the line' (P82), was enough to deter them from donating if a two-step approach is implemented.

Invalidating who they are

Asking a two-step question was viewed by some participants as invalidating who they are because they interpreted asking SAAB as suggesting that their gender is not sufficient or a 'real' reflection of who they are. Several likened it to asking racialized people where they are 'really from' when they say they are from Canada; a question that is widely understood by many in the Canadian context to be inappropriate, if not racist.

Underlying assumptions

Key to understanding the different views on a two-step question were assumptions held by participants regarding the need for blood operators to know both gender and SAAB. Those who were in favour of a two-step question did not question why the blood operator needs to know both. Some stated that they understood the blood operator needs to know SAAB, and many spoke about preferring a two-step question over the single question asking a donor's gender followed by additional questions to 2STGD donors only (i.e., the screening process at the time of the study). This suggests that participants in favour held the assumption that blood operators need to know both gender and SAAB. Most participants who were not in favour or uncertain of a two-step question questioned why blood operators would need to know SAAB, suggesting that they did not assume that blood operators need this information. For example:

> I don't want to just randomly disclose to people my sex assigned at birth. I don't have to do that in literally any other setting… Even in a healthcare setting, I don't need to randomly disclose my transness unless it's related to my care, right? If I have a uterus, it's only relevant if I have abdominal pain. If I'm going in for a sprained ankle, who cares?… If you're taking my blood, why are you asking me about my genitals?
>
> (P85)

Several suggested that a better approach than a two-step question would be to continue to move to a more gender-neutral screening process that relies less on organizing donors into different genders to assess donor health, recipient safety, and blood/plasma processing. Instead, participants preferred the blood operator to ask all donors about specific information needed. For example, asking all donors about pregnancies rather than asking only those donors registered as woman/female. As one participant put it, asking directly is better than 'approaching it [i.e., needed information] sideways' (P29).

Expanding gender options

All participants were in favour of expanding gender options beyond binary options if the blood operator needs to know a donor's gender. Several explained that expanding options would provide visibility and validation for 2STGD donors without having to ask a two-step question. At minimum, participants recommended adding 'nonbinary', 'Two-Spirit' and 'self-describe' options. Because genders are dynamic and may change, they suggested that a 'self-describe' option, or an option that would enable donors to write in their gender, would be preferred. Several suggested 'prefer not to say' as an option to make gender disclosure optional.

DISCUSSION

2STGD donors' views on more inclusive ways to register gender offer important insights for blood operators. First, taken as a whole, we suggest that results indicate that a two-step approach for all donors is not recommended unless it is imperative that the blood operator know both a donor's gender and SAAB to ensure donor and/or recipient safety. This suggests that recommendations for a two-step approach in health and population surveys are not necessarily transferable to a donation context. Given that participants in favour of a two-step approach assumed that blood operators must know SAAB, the imperative to know SAAB must first be established, and the need to know SAAB must be weighed against participants' concerns regarding potential psychological and physical harm of asking SAAB.

On the question of whether it is imperative to know SAAB, consideration of the uses of gender/SAAB in blood donation is warranted. For blood operators who have implemented an SBBS approach, a donor's gender/SAAB is no longer needed to determine which version of the gendered MSM question they must answer. If MSM questions remain, it is necessary to consider whether knowing a donor's SAAB is imperative, or if the information to determine which version of the question to ask could be arrived at differently. For blood operators who use gender/SAAB to ask only those registered as W/F about prior pregnancies and miscarriages to guide blood component use, they might consider asking all donors this question. Many participants preferred that blood operators ask direct questions to elicit the information they need rather than using gender/SAAB as a proxy. Use of gender and/or SAAB to determine donor safety measures should also be considered. Many 2STGD, and cisgender, donors take hormones that can impact haemoglobin level [20] and may also impact EBV [3]. As such, using a donor's SAAB may not offer the most accurate donor safety measures, and asking about hormone use may be warranted.

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While not an exhaustive list, these considerations are offered to support blood operators in different jurisdictions and contexts that may have different practices.

If, after having considered all options, blood operators consider it necessary to ask a two-step question, it is imperative that they consider how the questions will be asked (e.g., online or in person, one time or every donation), how the information will be stored, who will have access to it, and how the information will be used (i.e., why gender and SAAB are necessary for blood donation) must be explained to donors. To minimize risks to psychological and physical safety, we recommend asking donors their SAAB online and only once, ensuring security of this information and providing access only to staff who require it for specific purposes. That is, minimizing the potential for a donor's SAAB to be shared in public areas and thereby compromise safety. If the questions are asked in person, staff must be trained to ensure questions are asked in a respectful and inclusive manner.

A second option is to ask all donors a single question regarding their gender and to expand gender options. All participants were in favour of this change, leading to our recommendation that gender options be expanded, even if a two-step approach is taken. Expanding gender options could also deliver on the desire of some participants to feel validated, make gender diversity visible, and to treat 2STGD and cisgender donors alike. In Canada, additional options should include nonbinary, Two-Spirit, and self-describe, which would enable donors to write in or tell staff their gender if they feel safe enough to do so. As some participants noted, an increasing number of health and bureaucratic forms now offer additional gender options, increasing the expectation that blood operators do the same. In Canada, the census now includes a third, open text, option under the question asking the respondent's gender [21]. For other countries, research in collaboration with 2STGD communities should be conducted to determine which additional gender options to include. Participants explained that expanding gender options would be most significant for nonbinary donors. We recognize that a significant limitation in providing additional gender options is the BECS used by many blood operators, and would encourage ongoing international collaboration to make necessary system updates. Updates should include flexibility in options to allow blood operators in different countries to offer options that are meaningful and appropriate for their context.

With additional gender options, we recommend ongoing research and review of existing research to apply donor safety measures and criteria (i.e., minimum haemoglobin, donation frequency, and EBV) to nonbinary donors. Review of research on physiological markers (e.g., ferritin, exogenous and endogenous hormone status), donor choice through an informed approach (i.e., providing information about donor safety measures associated with each gender option so that donors can make an informed decision when registering their gender), or other ways to determine donor safety measures for nonbinary donors should be conducted to establish the most accurate and inclusive donor screening criteria. Nonbinary donors may be on hormone therapy [22] and therefore blood operators should not assume that they are not when determining appropriate donor safety measures. In the shorter term, while research and evidence synthesis is ongoing, the Association for the Advancement of Blood and Biotherapies recommends applying 'the most protective donor safety measures for nonbinary people' [23]. These criteria, however, should not be referred to as binary gender/SAAB criteria (e.g., referred to as applying the 'female criteria' to nonbinary donors). Rhetorical naming plays a significant role in organizing systems of thought that can reinforce cisnormativity and contribute to erasure of nonbinary people.

A third option is for blood operators to move towards a more gender-neutral donation process whereby knowing a donor's gender may be optional. This is already happening with, for example, the move to a more gender-neutral SBBS approach by some blood operators. Asking all donors about prior pregnancies is another example of providing a more gender-neutral process. That some participants suggested donors should have the option to provide their gender indicates some support for a fully gender-neutral process. On the other hand, other participants valued being seen as 2STGD and making gender diversity among donors visible. For many of these donors, blood/plasma donation was an act of social advocacy, demonstrating that 2STGD people participate in acts of social citizenship. While efforts to offer a more gender-neutral donation process are ongoing, we recommend that reasons for asking gender be explained, gender options be expanded, measures associated with gender options be clearly explained, and to enable donors to indicate their gender online/on an app.

Finally, staff must be trained to engage with donors on this topic. At the very least, staff training should equip them to explain clearly why gender (and/or SAAB) is relevant to donation, how the information will be used and who will have access to this information. Many participants did not know why this information was needed, and/or were given conflicting information. Frontline staff are critical to ensuring a positive donation experience [24] and ensuring safety in the donor centre [25].

2STGD donors make valuable contributions to blood systems and blood operators should continue their efforts to provide a more inclusive donation experience for them. This paper contributes evidence-informed recommendations to support blood operators in these efforts. Ongoing research and engagement with 2STGD donors is needed to address multiple barriers that they experience during donation.

Limitations

To build on thematic findings, the prevalence of differing views could be determined by conducting a survey with a larger representative sample of 2STGD donors. Our study sample had an overrepresentation of experienced and White donors whose views may differ from first-time and racialized donors. Further study of first-time and racialized 2STGD donors is needed with an intersectional analysis. Finally, this study was conducted before changes made to the trans screening criteria in September 2022. Examining the impact of these changes on 2STGD donors is highly recommended.

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J.H. contributed to conceptual design of the study, conducting the research and analysis, writing the first draft of the manuscript and revisions; T.B.F. and B.M. contributed to conceptual design of the study, data collection and revisions of the manuscript; D.L., J.B., J.E. and C.J. contributed to conceptual design of the study and revisions of the manuscript; S.G.G. contributed to data collection and analysis, and revisions of the manuscript; and A.D. contributed leadership on the conceptual design of the study, data analysis and revisions of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are not publicly available.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Red cell concentrates from teen male donors contain poor-quality biologically older cells

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Abstract

Background and Objectives: Donor factors influence the quality characteristics of red cell concentrates (RCCs) and the lesions that develop in these heterogeneous blood products during hypothermic storage. Teen male donors' RCCs contain elevated levels of biologically old red blood cells (RBCs). The aim of this study was to interrogate the quality of units of different donor ages and sexes to unravel the complex interplay between donor characteristics, long-term cold storage and, for the first time, RBC biological age.

Materials and Methods: RCCs from teen males, teen females, senior males and senior females were density-separated into less-dense/young (Y-RBCs) and dense/old RBCs (O-RBCs) throughout hypothermic storage for testing. The unseparated and density-separated cells were tested for haematological parameters, stress (oxidative and osmotic) haemolysis and oxygen affinity (p50).

Results: The O-RBCs obtained from teen donor samples, particularly males, had smaller mean corpuscular volumes and higher mean corpuscular haemoglobin concentrations. While biological age did not significantly affect oxygen affinity, biologically aged O-RBCs from stored RCCs exhibited increased oxidative haemolysis and decreased osmotic fragility, with teenage male RCCs exhibiting the highest propensity to haemolyse.

Conclusion: Previously, donor age and sex were shown to have an impact on the biological age distribution of RBCs within RCCs. Herein, we demonstrated that RBC biological age, particularly O-RBCs, which are found more prevalently in male teens, to be a driving factor of several aspects of poor blood product quality. This study

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emphasizes that donor factors should continue to be considered for their potential impacts on transfusion outcomes.

Keywords
donor factors, RBC biological age profiling, RBC quality testing

Highlights

- Red blood cell (RBC) heterogeneity, as defined by differences in cellular density and biological age, joins donor characteristics as a main contributor to RBC storage lesions.
- Biologically old RBCs from teenage males are smaller, possess an increased amount of haemoglobin and more readily haemolyse in the presence of oxidative stress.
- The observed decrease in the quality of red cell products from teen male donors over the entirety of storage is attributable to the increased proportion of biologically old RBCs in these red cell units.

INTRODUCTION

With over 118 million blood products collected globally for use in transfusions, the quality of the blood products is important for safe and favourable outcomes [1–5]. Red cell concentrates (RCCs) can typically be stored up to 42 days across most jurisdictions [6]. Beyond this storage time, blood products are considered unsafe for clinical use as a protective measure against well-established storage lesion accumulation [1, 7, 8]. Storage lesions refer to the physical, chemical and metabolic changes in donated blood over time that ultimately result in increased susceptibility to haemolysis during transfusion [1, 3, 5]. As the ageing of red blood cells (RBCs) during blood bank storage alone has failed to fully explain adverse transfusion outcomes, research has been aimed at understanding how donor properties and biological ageing (the ratio of recently matured to senescent cells) impact RBC products [9, 10]. A growing number of studies have found correlations between storage time and donor factors on patient outcomes with the fluctuations in RBC characteristics thought to contribute substantially to negative transfusion events [1–3, 5]. A better understanding of how the biological age of RBCs at the time of donation impacts RCC quality may help improve cell quality throughout storage and, in turn, impact post-transfusion reactions [11, 12].

The inherent differences among blood donors makes variability between blood products inevitable [13–16]. The heterogeneity of RCCs observed from different donors can be attributed to differential erythropoiesis caused by age, hormones, anaemia, female menstruation, erythrophagocytosis, donation frequency, exercise, and altitude exposure [13, 17–21]. These factors alter the distribution of recently matured, young RBCs (Y-RBCs) and senescent, old RBCs (O-RBCs) in a donated blood product [13, 17]. For nearly four decades, differential density fractionation of RBCs has been conducted, establishing that with increasing cellular density, RBC biological age also rises [22–24]. Ageing of RBCs both biologically and during storage is characterized by morphological changes, wherein they evolve from biconcave discocytes/smooth discs to spherocytes/smooth spheres [17]. RBCs also experience ex vivo changes in their metabolic activity (such as decreases in 2,3-diphosphoglyceric acid [2,3-DPG] levels) and intracellular composition (increasing reactive oxygen species) [13, 17, 25, 26]. While RBC storage lesion trends in the context of donor groups alone have been previously studied, the impact of RBC subpopulations together with the influence of donor factors such as age and sex has not yet been researched extensively. Recently, by employing a series of increasing Percoll densities, we separated Y- and O-RBCs, which enabled us to quantify estimated median densities (EMD) [27]. In Mykhailova et al. [27], we defined the EMD as the Percoll density wherein less-dense and more-dense RBCs were found at equal proportions. Assessment of RCCs derived from teen and senior donors of each sex revealed units from teenage males to have the highest amount of biologically old cells as reflected by their elevated EMDs [27].

This work was conducted to assess the contribution of RBC heterogeneity to previously observed donor-dependent RBC quality dynamics over storage time. With this study, we determined the impact of RBC biological ageing on several aspects of RCC storage quality through performance of haematological parameter testing, stress-induced haemolysis assays and profiling of oxygen affinity. We hypothesized that blood from teenage males would be more susceptible to external environmental stressors, due to inherent biological differences and the higher amounts of old RBCs within their donated units.

MATERIALS AND METHODS

Blood collection

This study was approved by the Canadian Blood Services Research Ethics Board. Sixty CPD/SAGM leuko-depleted (LD) RCCs from consenting donors were produced by the red cell filtration (RCF) method at Canadian Blood Services (Calgary, AB, Canada) (Table S1). Teenage (17–19 years old) and senior (>75 years old) donors were separated by sex: ($n = 15$) teenage males, ($n = 15$) teenage females, ($n = 15$)

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senior males and ($n = 15$) senior females. Available units in inventory were reviewed to select those that originated from donors from the requisite age and sex groups (Table S1). All RCCs were obtained within 24 h of LD and were stored for 42 days (1–6°C) in a monitored refrigerator between testing days. The RCCs all met the Canadian Standards Association quality control criteria for transfusable blood products.

Biological age profiling of RCCs by Percoll density gradient centrifugation

To isolate portions of young, less-dense RBCs from old, more-dense RBCs, Percoll (Percoll® GE Healthcare, Sigma-Aldrich, St. Louis, MO, USA) density gradient centrifugation was performed as previously described on four occasions [day 5, 14, 28 and 42 (±3 days)] during hypothermic storage [27]. Briefly, a panel of eight solutions were chosen based on initial donor RCCs' mean corpuscular haemoglobin concentration (MCHC) values and typically ranged in Percoll density from 1.083 to 1.107 g/mL (Figure S1). The resulting subpopulation samples were adjusted to a haematocrit between 40% and 55%, the final volumes measured using a calibrated pipette and the isolated RBC portions calculated by multiplying the measured volume by the RBC concentration. We designated the top (26.1 ± 7.5%) and bottom (18.8 ± 8.5%) portions of RBCs as Y-RBCs and O-RBCs, respectively, and considered these subpopulations to consist purely of cells at the extreme ends of biological ageing (Figure S1). On each testing day, a population of unseparated RBCs (U-RBCs) was also retained as a control group.

RBC quality assessment

A haematology analyser (DxH 520, Beckman Coulter, Co. Clare, IE) was used to obtain RBC count and index measurements for the following parameters: MCHC and mean corpuscular volume (MCV). Haematocrit measurements were obtained by centrifugation (14,848×*g*, 5 min, 20°C) of sample-filled capillary tubes and subsequent visual inspection (Haematokrit 210, Andreas Hettich GmbH & Co., Tuttlingen, DE). Storage haemolysis of U-RBCs was determined with the Drabkin's method of quantifying total and supernatant haemoglobin (Hb) concentrations [28, 29]. Oxygen affinity was assessed using an automated blood-oxygen analyser (Hemox-Analyser Model B, TCS Scientific, New Hope, USA) [17, 30, 31].

RBC stress-induced haemolysis measurements

Phosphate buffered saline (PBS)-washed U-, Y- and O-RBC samples were assessed for oxidative and osmotic haemolysis at each time point throughout hypothermic storage. Oxidative haemolysis was determined using a method wherein RBCs were incubated with a 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution at a final concentration of 150 mM [32, 33]. Osmotic haemolysis was induced by incubating RBC subpopulations in a hypotonic, glycerol-containing Bis–Tris-based buffer [32, 34].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism v.9.4.1 (GraphPad Software Inc., La Jolla, CA, USA). Mixed model analysis for repeated measurements with multiple comparisons was performed in order to estimate the effects of subpopulation (U-RBCs, Y-RBCs and O-RBCs), sex (female or male), age (teen or senior), group (teen male [TM], teen female [TF], senior female [SF] and senior male [SM]) and storage time (5, 14, 28, 42 days) and their various interactions.

RESULTS

Compared with females, unseparated RBCs from males possess elevated haematocrits in addition to a significantly higher propensity to haemolyse late into cold storage

Consistent with previously published data, a strong association between RBC haemolysis and the time spent in hypothermic storage was found when U-RBCs from all donor groups were tested (Figure 1a,b) [17]. Particularly, at day 42, RBCs from male donors possessed significantly increased haemolysis compared with the female donors ($p = 0.0092$) (Figure 1b). Although there were no donor age-dependent differences in haematocrit measurements observed in this study, sex-based changes have been reported in the past and indeed the haematocrit values found for U-RBCs from male units were consistently higher over the course of cold storage ($p \leq 0.0001$) (Figure 1c,d) [35].

Stored RBCs of different biological ages exhibit alterations in haematological indices based on donor age and sex

Having previously validated our method to biologically age profile RBCs based on their cellular density, we found that RBCs from senior donors, regardless of sex and biological age, were significantly larger in volume when compared with teenage RBCs ($p \leq 0.05$) (Figure 2) [27]. We next measured the MCHC levels of the unseparated donor samples and the prepared RBC subpopulations. The MCHC of O-RBCs was significantly higher than U-RBCs and Y-RBCs ($p \leq 0.0001$), whereas Y-RBCs had a significantly lower MCHC compared to the two other RBC subpopulations at day 5, 14 and 28 ($p < 0.01$) (Figure 3a). Upon age and sex stratification, U-RBCs from the TM group had a significantly higher MCHC compared with SF on all days of testing ($p \leq 0.003$) and TF only on days 5 and 28 ($p \leq 0.04$) (Figure 3b). Examination of the Y-RBC subpopulation

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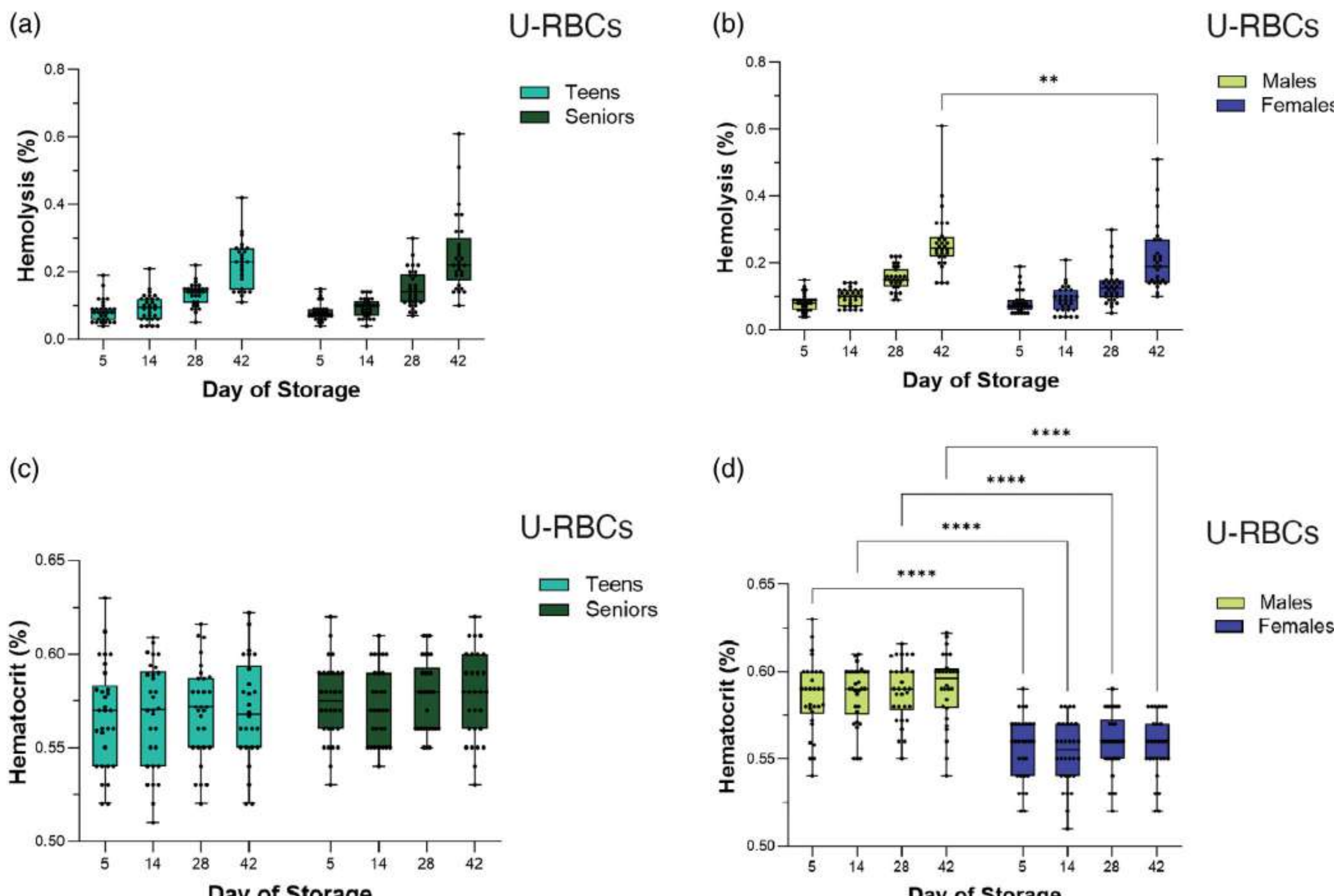

FIGURE 1 Haemolysis and haematocrit of unseparated red blood cells (U-RBCs) stratified by donor age or sex. Haemolysis measurements of U-RBCs shown as (a) age- or (b) sex-stratified. Haematocrit measurements of U-RBCs shown as (a) age- or (b) sex-stratified. Multiple comparisons tests were used to show significant differences (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, the preceding sentence was corrected.]

revealed that TM had significantly higher MCHC values compared with SF on storage day 5 and both female groups on day 42 of storage ($p \leq 0.03$) (Figure 3c). Like their Y-RBC and U-RBC counterparts, O-RBCs originating from TM donor units also had significantly higher MCHC compared with SFs on day 5 and day 42 ($p \leq 0.01$) (Figure 3d). On day 28, however, both male groups exhibited significantly higher MCHC levels than either female group ($p \leq 0.05$) (Figure 2d). Male RBCs, regardless of biological age, had significantly higher MCHC values than female RBCs across hypothermic storage ($p \leq 0.05$) (Figure S2).

O-RBCs exhibit higher levels of oxidative haemolysis throughout hypothermic storage in an age- and sex-dependent manner

O-RBCs were significantly more susceptible to oxidative haemolysis than U-RBCs and Y-RBCs ($p \leq 0.01$) (Figure 4a). Stratification of oxidative haemolysis results by age and sex revealed that TM exhibit the highest levels of oxidative haemolysis, followed by TF, with the lowest results being observed with senior donors (Figure 4). All TM donor RBCs, regardless of density separation, demonstrated increased AAPH-induced oxidative haemolysis compared with both senior groups across the entirety of the storage period ($p \leq 0.05$) (Figure 4b–d). When Y-RBCs and O-RBCs from teenage males were compared with the TF group on day 28, a significant difference in oxidative haemolysis was found ($p \leq 0.05$) (Figure 4c,d). Considering the RBC subpopulations in isolation, age rather than sex was the most significant modifier of oxidative haemolysis (Figure S3). At every testing point during hypothermic storage, U-, O- and Y-RBCs from teen donors exhibited a higher propensity to lyse under oxidative stress ($p \leq 0.04$) (Figure S3).

Osmotic haemolysis of RBCs throughout cold storage is most significantly impacted by sex and biological age

Regarding RBCs ability to withstand alterations in osmolality in the context of donor age and sex, assessment of osmotic haemolysis was carried out on unseparated and density-separated donor RBC products over storage. When analysing data in aggregate from all donors,

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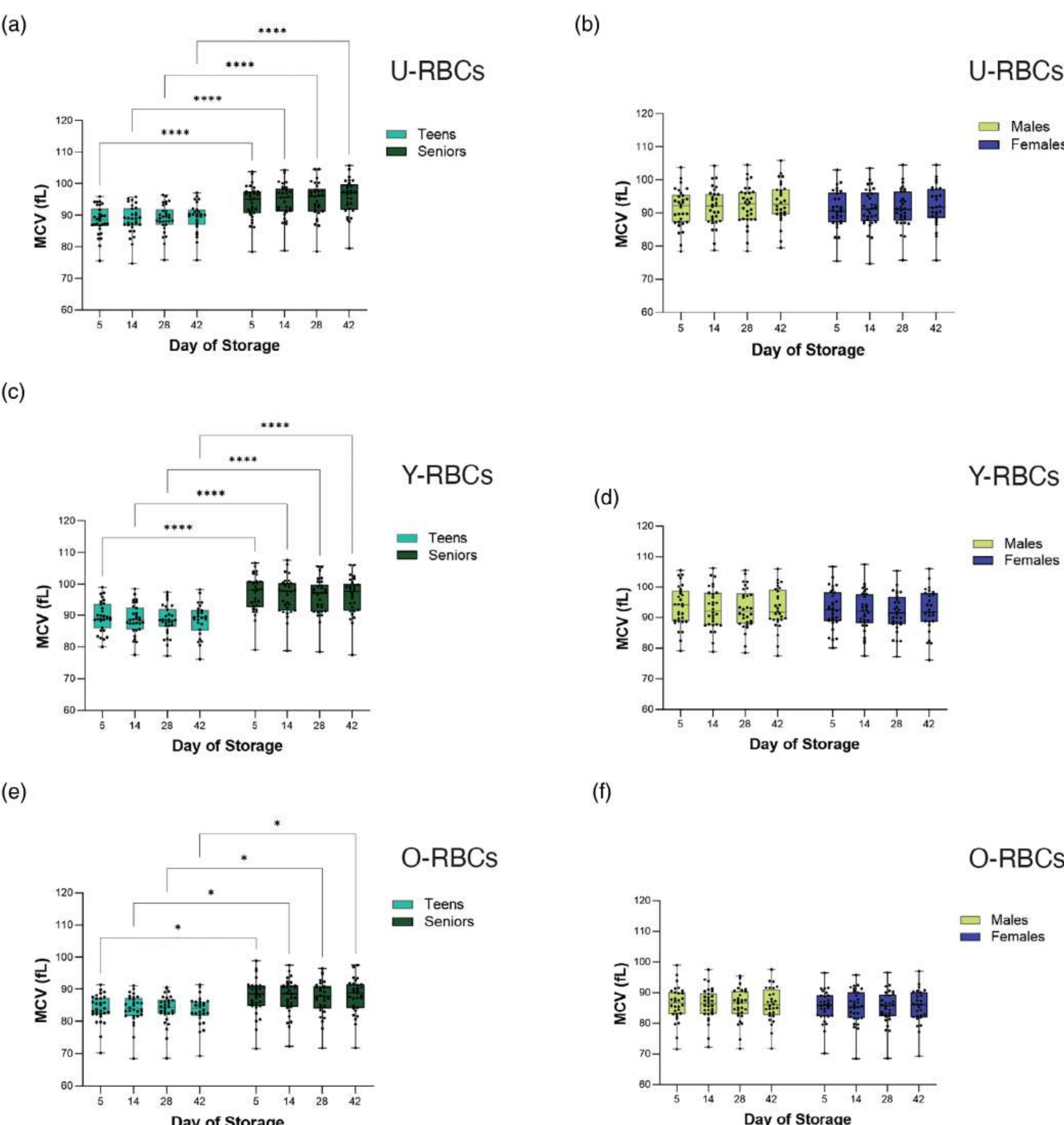

FIGURE 2 Mean corpuscular volume (MCV) of unseparated red blood cells (U-RBCs), young RBCs (Y-RBCs) and old RBCs (O-RBCs) stratified by donor age or sex. MCV measurements shown as age- or sex-stratified for (a,b) U-RBCs, (c,d) Y-RBCs and (d,e) O-RBCs. Multiple comparisons tests were used to show significant differences (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, the preceding sentence was corrected.]

O-RBCs compared to their U- and Y-RBC counterparts were less susceptible to osmotic haemolysis throughout the 42 days of storage ($p \leq 0.01$) (Figure 5a). Complete donor stratification showed that, specifically, TM donors within their U-RBC and Y-RBC subpopulations had significantly higher percent osmotic haemolysis at day 42 of storage compared with both TF and SF ($p \leq 0.05$) (Figure 5b,c). Notably, O-RBCs did not demonstrate an association between donor characteristics and a change in osmotic haemolysis over hypothermic storage time (Figure 5d). While age stratification of osmotic haemolysis data revealed no significant differences between teens and seniors, sex stratification of the osmotic haemolysis results led to the observation that male U-, and Y-RBCs had significantly higher osmotic

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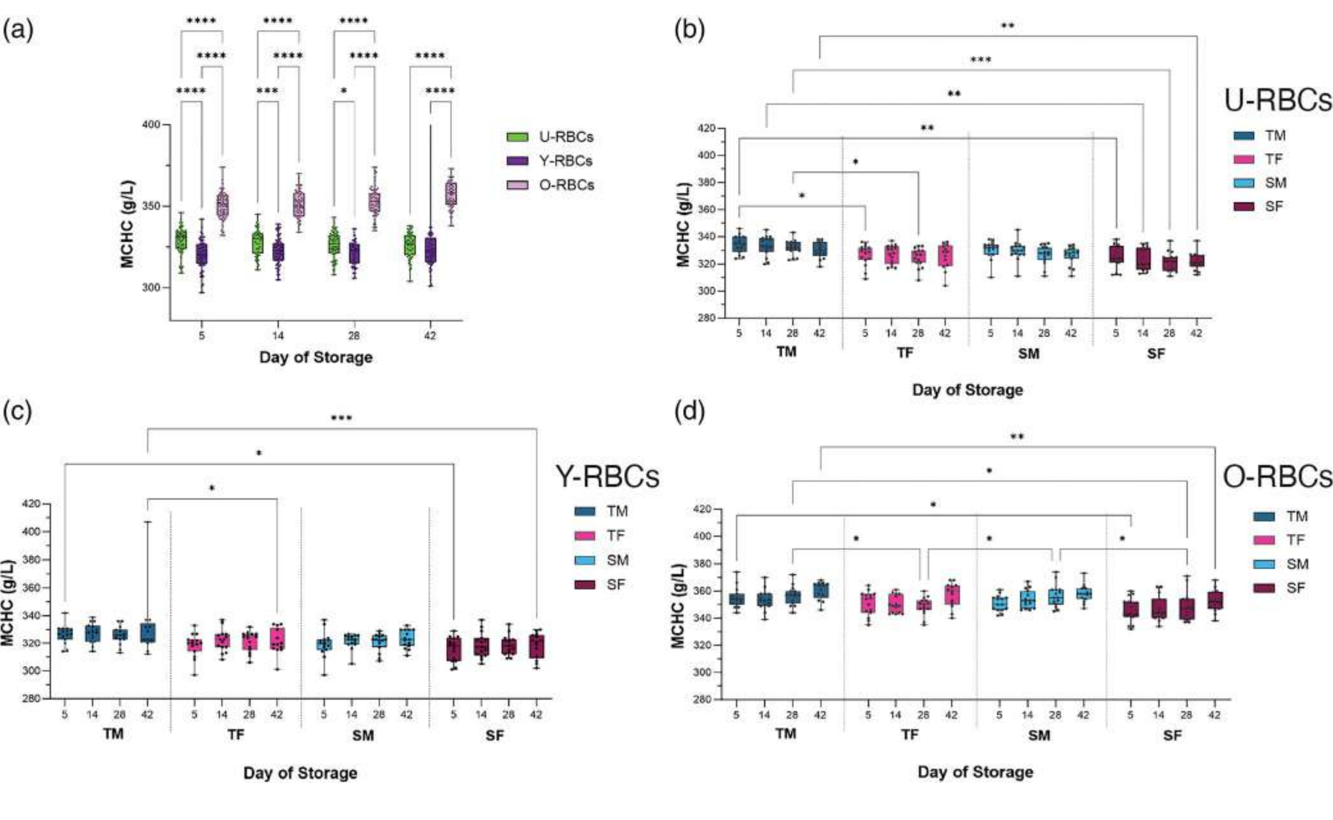

FIGURE 3 Mean corpuscular haemoglobin concentration (MCHC) of unseparated red blood cells (U-RBCs), young RBCs (Y-RBCs) and old RBCs (O-RBCs) stratified by donor age and sex. (a) The MCHC measurements for the three populations of RBCs (U-RBCs, Y-RBCs and O-RBCs) examined without any stratification based on donor characteristics. MCHC data shown as age- and sex-stratified for (b) U-RBCs, (c) Y-RBCs, and (d) O-RBCs. Multiple comparisons tests were used to show significant differences (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, Figure 3 and the preceding sentence were corrected.]

haemolysis levels compared with female donors on day 42 ($p \le 0.01$) (Figure S4).

The haemoglobin oxygen affinity of density separated RBCs throughout cold storage is most significantly influenced by age

While haemoglobin oxygen affinity measurements had high variability at all testing timepoints for all biological age subpopulations, the median oxygen affinities of these stored RBCs ranged between 16 and 20 mmHg, only slightly lower than what has been measured under normal conditions for humans (Figure 6a). Donor age imparted the most significant effect on this RBC quality parameter ($p \le 0.03$) (Figure S5). On day 42, all the U-, Y- and O-RBC subpopulations from teen donors exhibited higher p50 values or lower oxygen affinities than the combined senior donor group ($p \le 0.006$) (Figure S5). Interestingly, when donors were stratified further by age and sex, TFs demonstrated higher p50 values on day 42 compared with SM and SF donors in U-RBCs ($p < 0.03$) and only SM donors in density-separated cells (Y-RBCs: $p = 0.005$; O-RBCs: $p = 0.02$) (Figure 6b–d). This finding contrasts with testing results on day 5 for U-RBCs and O-RBCs which found that SM donor RBCs had a p50 value significantly higher than teen donors of both sexes ($p \le 0.01$) (Figure 6b,d).

DISCUSSION

This study provided an in-depth assessment of the quality of biologically young and old RBC subpopulations, their susceptibility to various storage lesions and associations to donor age and sex (Table 1). We employed our previously reported methodology of Percoll density-based separation, to isolate RBCs of various biological ages from donor samples at the extremes of the donor age spectrum [27]. We demonstrated teen males' RCCs to have more of the distinguishing biochemical and physical changes associated with the storage lesion that in turn impair the ability of their RBCs to adapt to external stresses (Table 1). Our results also further implicate the high levels of biological old cells within the blood products of male teens as one of potential contributors to their poorer storage quality.

Concurrent with other recent research efforts, teenage donors in this study demonstrated decreased MCVs relative to senior donors and increased Hb levels compared with female donors [36]. MCV typically increases with donor age; therefore, teens are expected to have smaller cell volumes than more senior donors [37]. Regardless of

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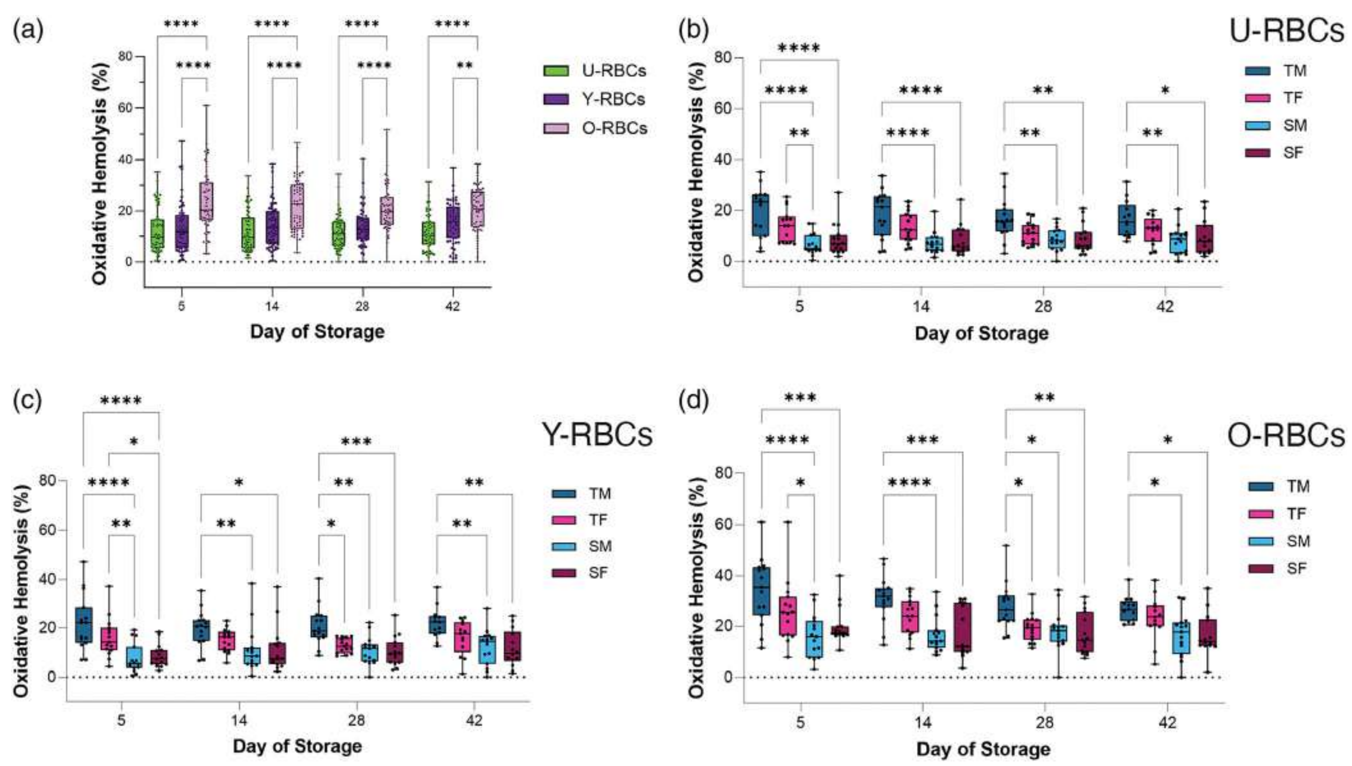

FIGURE 4 Oxidative stress-induced haemolysis of unseparated red blood cells (U-RBCs), young RBCs (Y-RBCs) and old RBCs (O-RBCs) stratified by donor age and sex. (a) Oxidative haemolysis reported as percentages for the three populations of RBCs (U-RBCs, Y-RBCs and O-RBCs) examined without any stratification based on donor characteristics. Outcomes of oxidative haemolysis assays shown as age and sex-stratified for (b) U-RBCs, (c) Y-RBCs and (d) O-RBCs. Multiple comparisons tests were used to show significant differences (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, Figure 4 and the preceding sentence were corrected.]

RBCs' biological age but to the greatest extent observed with O-RBCs, blood from TM especially exhibited an increased MCHC, which can be attributed to the increased levels of Hb together with lower MCV. The increased Hb levels in young men can be ascribed to sex hormones in males, as androgens typically enhance erythropoiesis [18]. Furthermore, in Mykhailova et al. [27], we found that young male donors' units have higher EMDs, another contributing factor to the lower MCVs witnessed in teen men. That haematological indices did not significantly decrease with successive weeks in storage is not surprising as other studies with extended storage times upwards of 6 weeks observed similar findings [38]. The inter-donor variability observed with the MCV measurements is probably caused by lifestyle factors (diet, alcohol consumption, smoking) and aspects of donor medical history that were not controlled for within this donor pool [39].

Oxidative haemolysis assays were conducted to measure the susceptibility of RBCs to haemolyse following exposure to oxidative stress as samples from donated units have been shown to become more prone to membrane peroxidation over time [26, 40, 41]. As expected, the Y-RBCs exhibited increased resistance to AAPH-mediated oxidative stress compared with O-RBCs. Oxidative haemolysis has been shown to decrease with donor age, so our observation of senior donors demonstrating more resistance to AAPH than teen donors is consistent with other publications that did not exclude large subsets of the elderly population based on their chronic conditions or various drug and supplementation regimens [32, 42]. While not apparent when our data were separated based on biological age, the effect of donor age on oxidative haemolysis is likely a reflection of seniors' lifetime donation frequency influencing their ferritin levels and subsequent erythropoiesis [13, 32]. Unfortunately, this level of donor information was not adequately captured within our study cohort. Osmotic haemolysis was used to assess the tolerance of RBCs to osmotic changes as age-based morphological transformations have been demonstrated to weaken cell membranes [43]. Unexpectedly, but likely in part due to the age profiling separation process, O-RBCs when compared with U- and Y-RBCs exhibited less osmotic haemolysis or in other words, demonstrated an increased resistance to osmotic stress that persisted and even further intensified with storage. Compared with their other biological age-separated counterparts, O-RBCs from male donors exhibited storage-dependent resistance to osmotic stress. The surface area to volume ratio changes associated with RBC biological ageing in TM may provide one explanation as to why but Cloutier et al. also showed the capacity of RBCs to shrink under hyperosmotic conditions (O_{Hyper} values) was significantly different with male donors [36]. The variability seen in the osmotic haemolysis

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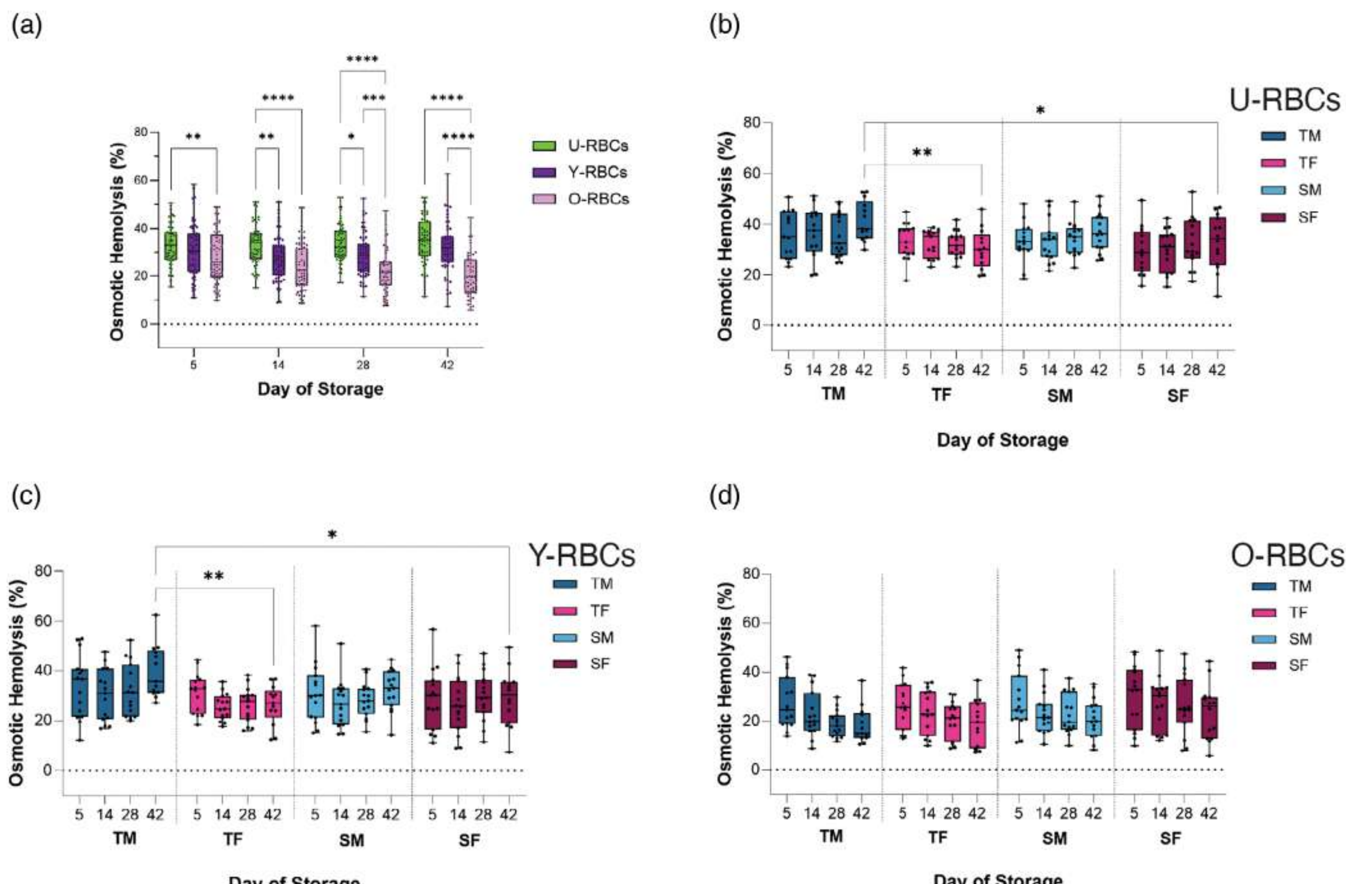

FIGURE 5 Osmotic stress-induced haemolysis of unseparated red blood cells (U-RBCs), young RBCs (Y-RBCs) and old RBCs (O-RBCs) stratified by donor age and sex. (a) Osmotic haemolysis reported as percentages for the three populations of RBCs (U-RBCs, Y-RBCs and O-RBCs) examined without any stratification based on donor characteristics. Outcomes of osmotic haemolysis assays shown as age- and sex-stratified for (b) U-RBCs, (c) Y-RBCs and (d) O-RBCs. Multiple comparisons tests were used to show significant differences (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, the preceding sentence was corrected.]

dataset also suggests that other donor factors beyond age and sex may need to be incorporated in the design of future studies [32]. In the past, Kanias et al. demonstrated osmotic haemolysis to be highly associated with donor race/ethnicity, which was a donor factor not considered within this current study [32].

As RBCs age, biologically and/or throughout storage, oxygen affinity tends to increase due to metabolic changes, particularly a decrease in the metabolite 2,3-DPG [25, 44, 45]. 2,3-DPG plays a vital role in the ability of stored RBCs to release oxygen [25]. Our results showed that SM donors possess the greatest p50 measurements, or the lowest affinities for oxygen, at day 5 across all cell subpopulations, whereas TF O-RBCs had increased p50 measurements near unit expiry at day 42. Teen donors have also been shown to have elevated levels of 2,3-DPG, relative to senior donors but in light of the findings reported here, donor factors effects on RBC metabolites warrants future reinvestigation in the context of biological age profiling [46, 47].

This study had several limitations, mainly that no middle-aged donors were evaluated and therefore, our interpretations regarding RBC biological age are limited to only the extremes of the adult population. The washing of Percoll density-separated RBCs in an isotonic solution (PBS) may also affect the outcome of the stress-induced haemolysis assays. Not only can significant RBC morphological changes take place upon incubation with PBS but PBS has also been found to increase susceptibility to osmotic haemolysis [48–50]. Since all our RBC subpopulations were PBS-washed, Y-RBCs could potentially have developed an increased sensitivity to osmotic pressure due to washing buffer-induced morphological changes compared with O-RBCs, which have already naturally progressed towards a more spherocytic shape.

In summary, we have demonstrated that the storage lesions observed in donated RCC units appear to be affected by their proportions of biologically mature RBCs. The increased density of RBCs from teen males closely corresponds with the lesions that their O-RBC subpopulations incur throughout storage. We demonstrated that biological age together with donor characteristics plays a pivotal role in altering the quality parameters of blood products, but the influence of our results on the outcome of blood transfusions remains unknown. Transfused units from teenage men, which contain elevated numbers

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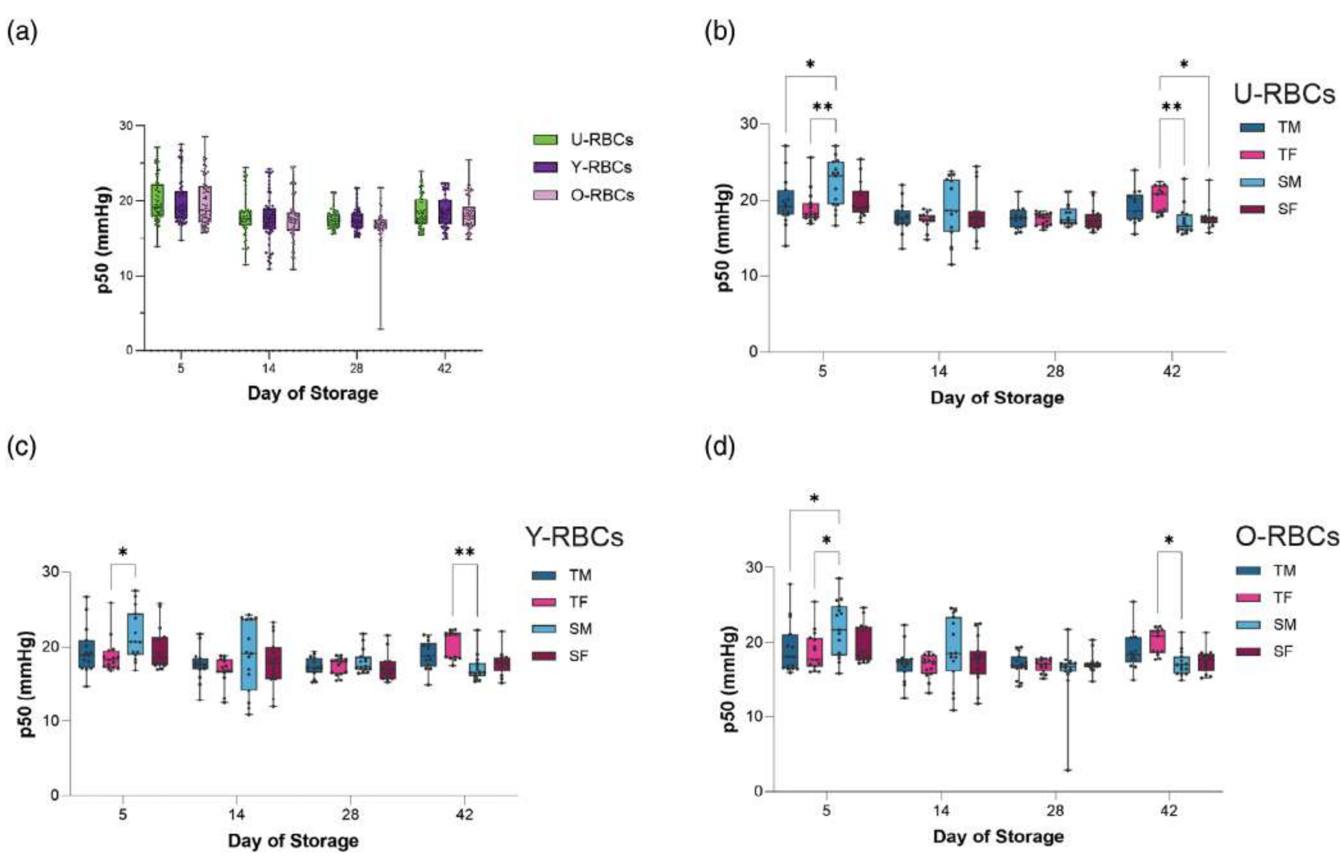

FIGURE 6 Oxygen affinities of unseparated red blood cells (U-RBCs), young RBCs (Y-RBCs) and old RBCs (O-RBCs) stratified by donor age and sex. (a) The p50 measurements for the three populations of RBCs (U-RBCs, Y-RBCs and O-RBCs) examined without any stratification based on donor characteristics. Oxygen affinities conveyed as age- and sex-stratified for (b) U-RBCs, (c) Y-RBCs and (d) O-RBCs. Multiple comparisons tests were used to show significant differences (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Medians are indicated as horizontal lines on the box and whisker plots. [Correction added on 28 March 2024: After first publication, the preceding sentence was corrected.]

TABLE 1 Summary of the observed data trends, with up and down arrows indicating increases and decreases in quality measurements respectively, and hashmarks corresponding to relatively unchanged values for that donor group in comparison to the other age- and sex-stratified groupings.

	Teens		Seniors		
Quality parameter	**Male**	**Female**	**Male**	**Female**	**RBC biological age**
MCV	↓	↓	↑	↑	U-, Y- and O-RBCs throughout storage
MCHC	↑	↓	–	↓	U-, Y- and O-RBCs throughout storage
Oxidative haemolysis	↑↑	↑	↓	↓	U-, Y- and O-RBCs throughout storage
Osmotic haemolysis	↑	↓	–	↓	U-, Y- but not O-RBCs throughout storage
p50	–	↓	↑	–	U-, O- but not Y-RBCs at day 5
p50	–	↑	↓	–	U-, Y- and O-RBCs at day 42

Abbreviations: MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; O-RBC, old red blood cells; U-RBC, unseparated red blood cells; and Y-RBC, young red blood cells.

of O-RBCs and whose O-RBCs are of smaller size, have a higher haemoglobin content and possess increased susceptibility over storage to haemolyse under oxidative stress could be more rapidly removed from circulation thereby dysregulating erythrophagocytosis [51–53]. The combination of increased senescent O-RBCs together with enhanced erythrophagocytosis, could challenge systemically inflamed transfusion recipients' immune responses thus leaving them potentially more prone to develop severe infections and sepsis [54, 55]. The work presented here supports the further assessment of the clinical consequences of the transfusion of senescent O-RBCs through the execution of studies using ex vivo human systems and pre-clinical animal models.

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O.M. was responsible for study design, data collection, data analysis and interpretation, in addition to drafting and reviewing the manuscript. M.B.C. was involved with data analysis and interpretation in addition to manuscript writing and reviewing. C.P. and M.Y. assisted with data collection and manuscript writing. C.O. assisted with study design, data collection, and manuscript reviewing. Q.L.Y. was involved with the analysis and interpretation of the data collected in this study. T.K. and J.A. were integral in study design, data interpretation, and manuscript reviewing. We acknowledge Rafay Osmani and April Xu for their assistance with data acquisition. We are grateful to Canadian Blood Services' blood donors who made this research possible.

[Corrections added on 28 March 2024: After first online publication, legends of all figures and supporting information as well as figures 3 and 4 were corrected.]

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Voluntary blood donation preferences in China: A discrete choice experiment among experienced and inexperienced donors

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Abstract

Background and Objectives: Due partly to an ageing population, China faces an increasingly dire blood shortage crisis requiring greater voluntary blood donations. A better understanding of blood donation preferences can inform blood donation policies and potentially increase donations. We used an online survey and discrete choice experiment to achieve our study objective: identify the most influential structural facilitators and barriers to voluntary blood donation in China.

Materials and Methods: First, we identified six structural attributes (travel time, venue, donation volume, paid leave, scheduling and gifts) that were hypothesized to influence voluntary blood donation; attribute selection was based on a literature review and qualitative interviews. Second, a d-efficient design with 36 choice sets and 9 blocks was developed. Participants were asked to complete four choice sets, and in each choice set, they were asked to choose from three options: two voluntary blood donation scenarios and a 'Do not donate blood' option. Study participants were recruited through an online survey platform company in China. Voluntary blood donation preferences and preferences by blood donation history were estimated with random-parameter logit models and interaction terms.

Results: In 2022, 1185 individuals enrolled in the study. Most participants had college education (92%). Generally, participants preferred longer paid leave, lower blood donation volumes and gifts after donation. Based on interaction analyses, experienced and inexperienced donors exhibited similar preferences.

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Conclusion: Campaigns to increase voluntary blood donation rates in China should consider implementing paid leave after voluntary blood donation, lower blood donation volumes and small gifts conferred after donation.

Keywords
altruism, Asia, blood donor, incentives, stated preferences

Highlights

- Due partly to an ageing population, China faces an increasingly dire blood shortage crisis, requiring greater voluntary blood donations.
- Results from this nationwide discrete choice experiment study indicate that small souvenir gifts, lower volumes of donated blood and paid leave for donors may be effective means of increasing voluntary blood donation rates in China.
- Pilot studies are needed to assess the efficacy and feasibility of implementing such interventions in real-world contexts.

INTRODUCTION

Blood donation practices in China have undergone major changes over the past 30 years. Up until the 1990s, commercial procurement of blood donation was relatively common [1–5], and nosocomial blood-borne disease infections were a serious public health challenge [1, 2, 4]. In response, the government passed the Law of the People's Republic of China on Blood donation in 1998, which prohibited paid blood donation [3, 4, 6] and promoted the establishment of related policies and public services [7]. The number of voluntary blood donors and the volume of blood donated nationwide achieved sustained growth for more than 20 years, with the rate of blood donation per 1000 people rising from 4.8 in 1998 to 11.5 in 2022. The total volume of blood donated in 2022 was 27.6 million units, with an average annual growth rate of 14.5% [8, 9], and the number of blood donors in 2022 exceeded 16 million, with an average annual growth rate of the number of blood donors of 16.3 percent [8, 9]. To date, approximately 453 blood stations have been constructed nationwide [9], and the level of blood security has greatly improved [10–16].

Despite progress in increasing voluntary nonremunerated blood donation, there remain serious challenges in maintaining an adequate blood supply in China. As China's population rapidly ages, current levels of voluntary blood donation will not be able to meet the rising demand in the future [6, 8–10]; the proportion of children (0–14) has dropped from 22.9% in 2000 to 17.95% in 2020, but the proportion of the senior population (over 65 years old) has increased from 7% to 13.5% in 2020 [17, 18]. According to forecasts from the United Nations, the share of the elderly population in China will increase to 48.3% by the year 2080 [12–14]. When the population ageing process intensifies, the ageing population will be less eligible to donate blood yet will require more blood transfusion due to higher incidence of disease afflictions and the need for more frequent medical procedures [19–21]. Generally, individuals over 55 years old are prohibited from donating blood in China, although in some areas, individuals over 55 may be able to donate blood if special medical exemptions are approved [22]. Hence, the demand for blood resources will increase due to the ageing problem.

Given the widening gap between blood supply and demand in China, it will be imperative to motivate people to donate blood voluntarily [6, 14, 15]. Understanding the role of non-monetary incentives may help inform the development of more effective blood donation policies [21, 23, 24]. In addition to individual motivators and beliefs about blood donation (e.g., wanting to help others, fear of health risks associated with blood donation) [16, 25], factors related to blood donation policies and services also appear to impact willingness to voluntarily donate blood. Such factors can include donation location, attitudes of venue staff and technical skills of the phlebotomists [14].

There is a large debate on the function of extrinsic incentives to motivate pro-social behaviours. Based on habit formation in behaviour and standard economic theory, extrinsic incentives can influence people's motivation positively. Research suggests that extrinsic incentives, especially economic incentives, such as cash and paid leave, inspire people to donate blood [21, 23, 26, 27]. However, other research indicates that extrinsic incentives would discourage people from donating because doing so would undermine intrinsic motivations [28, 29]. In China, financial rewards for donating blood are illegal [3, 30], and providing paid leave is recommended by local regulations [22, 31–35]. There have been claims that gifts are deemed more important than non-monetary incentives such as travel time [12], but there has been scanty empirical evidence about the influence of gifts and paid leave on blood donation preferences in China.

Discrete choice experiments

Discrete Choice Experiments (DCE) are an approach often used to elucidate individual health preferences [36–39]. The DCE presents competing hypothetical scenarios of a product or service and asks participants to state their preferred scenario. Health preference utilities and the relative importance of attributes and levels of a scenario can then be estimated quantitatively [37, 39]. DCE results can then shed light on which policy interventions will be most likely to resonate with the target population.

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In response to the urgent need for increased voluntary blood donation in China and the aforementioned concerns, we conducted a nationwide DCE to elucidate voluntary blood donation preferences in China.

METHOD

The DCE consisted of three stages: (1) Identification of blood donation attributes and levels, (2) DCE and questionnaire design and (3) Implementation of DCE.

Determination of attributes and levels

DCE attributes and levels were determined by literature reviews and conducting 11 qualitative interviews (5 males and 6 females, 18–60 years old) that focused on the factors influencing people's decisions to voluntarily donate blood. Keywords used in the literature search are enumerated in the Supporting Information (S2). Then, we recruited qualitative interview participants from a separate larger national survey of blood donation. Qualitative interviewees were asked to discuss their donation experience, the causes of their

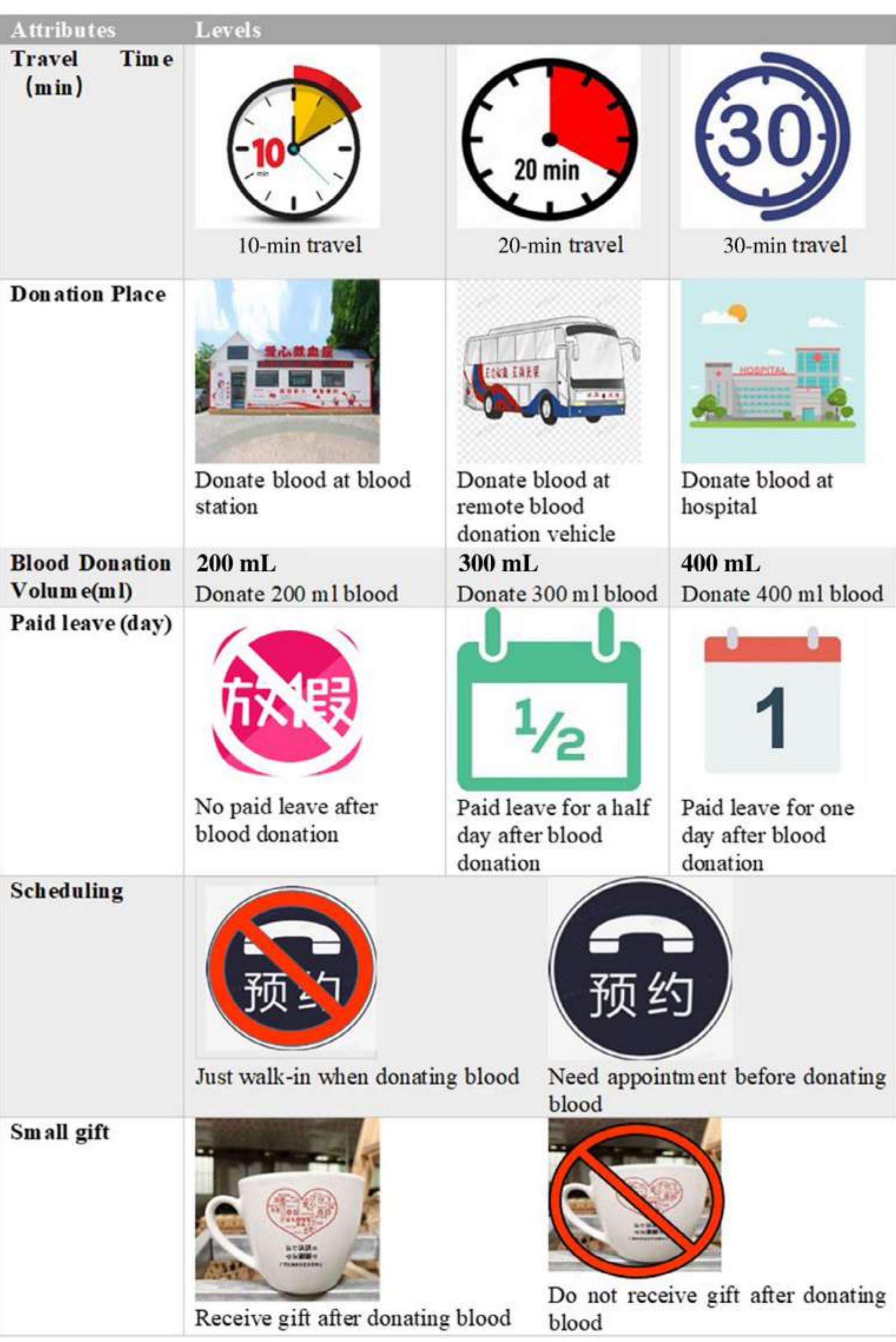

FIGURE 1 Attributes and levels of the discrete choice experiment.

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willingness or unwillingness to donate and to comment on attributes of blood donation service institutions. Qualitative interview participants received a cell phone credit worth 100 RMB (∼17 USD). Qualitative interviews were recorded, transcribed and analysed thematically [40]. Illustrative quotes from the qualitative interviews are presented in the Supporting Information (S4). Based on our literature review and qualitative interviews, we finalized six attributes and 16 associated levels for the DCE (Figure 1).

DCE design

A d-efficient design matrix with 36 different choice sets across nine blocks was developed with NGENE [21, 41]. There were four different choice sets in each of the nine blocks, and each participant was randomly assigned to a block by the online survey platform. Study participants were required to complete four choice sets, and in each choice set, they were instructed to choose from three options: two voluntary blood donation scenarios (options A and B) and the 'Do not donate blood' option. Previous studies have found that the format of the presentation in the choice experiment has an impact on the result of the choice [42–44]. To increase the cognitive understanding of the attribute levels, attribute levels were presented with both text and a representative image [44]. An example of a choice set is shown in Figure 2. The number of choice sets assigned to each participant was partly determined by consideration of potential survey fatigue.

DCE implementation

The DCE survey was conducted in August 2022. Eligibility criteria were being over 18 years old and currently residing in mainland China. Participants were recruited by an established online survey company Sojump (a Chinese platform for conducting surveys). Sojump draws from a pool of over 10 million respondents and is a popular participant recruitment platform used by Chinese social science researchers [45]. To estimate the required sample size, we used the following equation, as has been recommended and used in previous DCEs:

Choice A	Choice B	Choice C
20-min travel	10-min travel	Do not donate blood
Donate blood at blood station	Donate blood at remote blood donation vehicle	
200 mL Donate 200 mL blood	400 mL Donate 400 mL blood	
No paid leave after blood donation	Paid leave for one day after blood donation	
Just walk in when donating blood	Need appointment before donating blood	
Receive gift after donating blood	Do not receive gift after donating blood	Set1

FIGURE 2 Example of a choice set.

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$$\frac{nta}{c} \geq 500,$$

where n is the sample size, t is the number of choice sets completed by each participant, a is the number of alternatives per task and c is the product of the two attributes with the largest numbers of levels [46]. Using this equation and based on the parameters of the DCE study design, we determined that the minimum sample size should be 375.

Statistical analysis

Data analysis proceeded in two stages. Multinomial logit (MNL) and mixed logit (MXL) models were used in the first stage. Mixed logit models account for normal distributions of preference heterogeneity between respondents [38, 39, 47]. The second stage used a MXL with interaction terms (MXL-I). Interaction terms were based on whether the participant had ever donated blood before. Interaction models were also used to estimate attribute relative importance by age, gender and income. All analyses were conducted in R with the mlogit package and 1000 Halton draws [47]. The relative importance of each attribute was calculated by dividing its parameter estimate range by the sum of parameter estimate ranges for all attributes, excluding the influence of the opt-out selection and error terms [38, 48]. Confidence intervals for attribute relative importance were calculated by deriving the standard error of each attribute's range given the variance and covariance of its levels, using the following formula:

$$\sqrt{[(\text{Variance of Level A}) + (\text{Variance of Level B}) + (2 \times \text{Covariance between Levels A and B})]}.$$

TABLE 1 Participant sociodemographics ($n = 1185$).

	Total *n* (%)	Ever donated blood (%)	Never donated blood (%)	*p*-Value	General adult population (%)
n	1185	668	517		
Age					
18–25 years	345 (29)	35	24	0.5	9.3
25–30 years	357 (30)	26	33		8.3
Over 30 years	483 (41)	38	43		82.4
Sex groups					
Male	505 (43)	46.6	37.5	0.4	51.1
Female	680 (57)	53.4	62.5		48.9
Education					
Middle school and below	36 (3)	1.6	4.9	0.8	62.3
High school	62 (5)	4.3	6.5		16.4
College and above	1087 (92)	94.1	88.6		19.3
Region					
Northeast	60 (5)	4.8	5.4	0.3	7
Eastern China	398 (34)	40.1	23.3		30
North China	187 (16)	15.7	15.9		12
Central China	162 (14)	9.2	20.7		15.9
Southern China	215 (18)	16.1	21.4		13.2
Southwest	115 (10)	9.8	9.6		14.6
Northwest	48 (4)	4.3	3.7		7.3
Marital status					
Married	738 (62)	69.6	52.8	0.8	72.7
Single	433 (37)	29.5	45.6		19.2
Divorced	10 (1)	0.6	1.2		2.4
Widowed	4 (0)	0.3	0.4		5.7
Income USD/month					
<419	243 (21)	14.7	28.0	0.7	83.6
419–1117	501 (42)	42.4	42.2		18
1117–2093	338 (29)	34.0	21.5		2.1
2093–2791	73 (6)	7.5	4.4		0.28
>2791	30 (3)	1.5	3.9		0.05

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The significance of differences in relative importance was determined by non-overlap of confidence intervals. Additional background information about DCE modelling is presented in the Supporting Information (S7).

Ethics approval

The study protocol was approved by the Ethics review committee of Xi'an Jiaotong-Liverpool University. (No.: ER-SCI-001000005932022 0413123309).

Consent to participate

Consent to participate was obtained from all study participants.

RESULTS

In August 2022, 1204 individuals clicked the survey link. Nineteen were excluded because they were not living in mainland China or were less than 18 years old. Despite the general age limitation of voluntary blood donation, people over 55 years old were enrolled in the study because of the possibility of obtaining exemptions [22]. Therefore, there was a total of 1185 valid participants who completed 4740 choice set tasks.

Participants' characteristics

Participants' characteristics are shown in Table 1. Generally, the participants were young (more than half are below 30 years old), with at least college-level education (92%), and married (62%). Overall, 56.4% of the sample had previously donated blood, and 2.1% of participants were over 55 years old. There were no significant sociodemographic differences by blood donation history.

MNL model

Table 2 displays participants' blood donation preferences based on the MNL analysis. Findings indicated preferences for paid leave, small gifts, lower travel time and lower blood donation volumes.

MXL model

Table 3 shows the outcome of the MXL analysis. Again, results indicated a greater preference for preferences for paid leave, small gifts, lower travel time and lower blood donation volumes. According to Table 3, each attribute included more than one level with a statistically significant standard deviation of coefficients, implying the heterogeneity of preferences for all attributes [38, 48, 49].

TABLE 2 Blood donation preferences in China (MNL model).

Attributes and levels	Coefficient	SE	Odds ratio (95% CI)
Travel time			
10 min	0.152***	0.027	1.16 (1.10–1.23)
20 min	−0.037	0.028	0.96 (0.91–1.02)
30 min	−0.115***	0.031	0.89 (0.84–0.95)
Donation place			
Blood station	−0.020	0.027	0.98 (0.93–1.03)
Remote blood donation vehicle	−0.019	0.027	0.98 (0.93–1.03)
Hospital	0.039	0.031	1.04 (0.98–1.10)
Blood donation volume			
200 mL	0.231***	0.028	1.26 (1.19–1.33)
300 mL	0.000	0.028	1.00 (0.95–1.06)
400 mL	−0.231***	0.031	0.79 (0.75–0.84)
Paid leave			
No paid leave	−0.750***	0.030	0.47 (0.45–0.50)
Half day	0.207***	0.027	1.23 (1.17–1.30)
1 day	0.543***	0.030	1.72 (1.62–1.83)
Scheduling			
Walk-in	0.035*	0.017	1.04 (1.00–1.07)
Appointment necessary	−0.035*	0.017	0.97 (0.93–1.00)
Gift after donation			
Have gift	0.246***	0.018	1.28 (1.23–1.32)
No gift	−0.246***	0.018	0.78 (0.75–0.81)
Non-random parameter			
Opt-out ASC	−0.705***	0.025	

Abbreviations: ASC, alternative-specific constant; CI, confidence interval; MNL, multinomial logit; SE, standard error.
*$p < 0.05$; ***$p < 0.001$.

MXL-I model

Table 4 shows the result of the MXL-I model. This model extends the MXL model by crossing all levels with the interaction term, ever donated blood. Results indicated that previous donors have a stronger preference for the blood station venue, compared with those who have never donated before. As expected, participants who had ever donated, were less likely to choose the do not donate option. Scaled blood donation preferences by donation history are presented in Figure 3, with larger values implying stronger preference.

Relative importance by sociodemographics

Tables in Supporting Information S8 present blood donation preferences across various levels of income, age, gender and donation history.

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TABLE 3 Blood donation preference in China (MXL model).

Attributes and levels	Coefficient	SE	StdD	StdDSE	Odds ratio (95% CI)
Travel time					
10 min	0.254***	0.043	0.303*	0.152	1.29 (1.18–1.40)
20 min	−0.060	0.042	0.001	1.432	0.94 (0.87–1.02)
30 min	−0.193***	0.080	0.006	1.42	0.82 (0.70–0.96)
Donation place					
Blood station	−0.035	0.041	0.400***	0.121	0.97 (0.89–1.05)
Remote blood donation vehicle	−0.007	0.040	0.185	0.214	0.99 (0.92–1.07)
Hospital	0.042	0.075	0.280*	0.130	1.04 (0.90–1.21)
Blood donation volume					
200 mL	0.381***	0.049	0.561***	0.107	1.46 (1.33–1.61)
300 mL	−0.007	0.041	−0.153	0.296	0.99 (0.92–1.08)
400 mL	−0.374***	0.092	0.110	0.322	0.69 (0.57–0.82)
Paid leave					
No paid leave	−1.202***	0.094	0.976***	0.106	0.30 (0.25–0.36)
Half day	0.336***	0.045	0.002	0.965	1.40 (1.28–1.53)
1 day	0.866***	0.134	0.250	0.157	2.38 (1.83–3.09)
Scheduling					
Walk-in	0.048*	0.026	0.378***	0.082	1.05 (1.00–1.10)
Appointment necessary	−0.048*	0.026	0.354***	0.074	0.95 (0.91–1.00)
Gift after donation					
Have gift	0.362***	0.036	0.382***	0.082	1.44 (1.34–1.54)
No gift	−0.362***	0.036	0.302***	0.083	0.70 (0.65–0.75)
Nonrandom parameter					
Opt-out ASC	−0.588***	0.031			

Abbreviations: ASC, alternative-specific constant; CI, confidence interval; MXL, mixed logit; SE, standard error; StdD, standard deviation; StdDSE, standard error of the standard deviation.
*$p < 0.05$; ***$p < 0.001$.

The relative importance of each attribute was similar across all three age categories. However, blood donation volume was significantly more important to females than males (20.5% vs. 14.5%). In addition, travel time was more important for highest income respondents versus lowest income respondents (12.7% vs. 6.9%).

DISCUSSION

Results from this online nationwide DCE revealed several key findings about voluntary blood donation preferences among experienced and inexperienced donors in China. To the best of our knowledge, the present study is the first in China to examine blood donation preferences among inexperienced blood donors.

First, paid leave was by far the most important blood donation service attribute among the attributes that we assessed. These results are consistent with research from Germany and the United States, which also indicate that paid leave is of great importance to donors [21]. There are several possible reasons for the large relative importance of paid leave. First, according to Chinese traditional medicine, losing blood could disturb the balance of your body and weaken energy [16]. Therefore, Chinese citizens may prefer to rest after 'losing energy'. Even those who do not subscribe to traditional Chinese medicine's principles may have serious concerns about blood donation causing anaemia and physical weakness, which may interfere with work performance [16]. Such concerns also appear supported by participants' relatively strong preference for donating smaller volumes of blood. In addition, previous studies with inexperienced donors have shown that fear of adverse health consequences is a major reason for not donating [25]. Second, paid leave to donate blood can also be viewed as an authorized respite from work, which some employees may welcome. The blood donation process for a single donor is unlikely to require a half or full working day, and the remaining time of paid leave could be used for other personal business or leisure. However, employment organizations may have limited incentives to provide paid time off to rest from blood donations, so creative policies may be needed. Local legislative action may be an effective means of encouraging paid leave for blood donation. For example, by the end of 2023, six provincial-level administrative regions (Hainan, Inner Mongolia, Sichuan, Guizhou, Yunnan and Qinghai) had enacted legislation to establish 1–3 days of days off for blood donation [22, 31–35].

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TABLE 4 Blood donation preferences in China (MXL-I model).

Attributes and levels	Coefficient	SE	StdD	SE
Travel time				
10 min	0.194**	0.06	0.288	0.16
20 min	−0.023	0.063	0.001	1.455
30 min	−0.171**	0.062	0.007	1.401
Donation place				
Blood station	−0.129*	0.062	0.377**	0.128
Remote blood donation vehicle	0.012	0.059	0.212	0.191
Hospital	0.117	0.061	0.292*	0.128
Blood donation volume				
200 mL	0.374***	0.066	0.563***	0.107
300 mL	−0.074	0.061	−0.137	0.329
400 mL	−0.300***	0.066	0.043	0.728
Paid leave				
No paid leave	−1.267***	0.105	0.983***	0.106
Half day	0.376***	0.062	0	0.978
1 day	0.891***	0.083	0.260	0.154
Scheduling				
Walk-in	0.039	0.039	0.378***	0.082
Appointment necessary	−0.039	0.023	0.357***	0.075
Gift after donation				
Have gift	0.372***	0.046	0.383***	0.082
No gift	−0.372***	0.032	0.303***	0.084
Nonrandom parameter				
Opt-out ASC	−0.306***	0.038		
Interaction terms				
Ever donated blood × 10 min	0.111	0.08		
Ever donated blood × 20 min	−0.063	0.084		
Ever donated blood × 30 min	−0.048	0.082		
Ever donated blood × blood station	0.168*	0.083		
Ever donated blood × remote blood donation vehicle	−0.035	0.08		
Ever donated blood × hospital	−0.132	0.082		
Ever donated blood × 200 mL	0.016	0.081		
Ever donated blood × 300 mL	0.111	0.082		
Ever donated blood × 400 mL	−0.127	0.083		
Ever donated blood × no paid leave	0.103	0.089		
Ever donated blood × half day	−0.065	0.078		
Ever donated blood × 1 day	−0.037	0.082		
Ever donated blood × walk-in	0.02	0.052		
Ever donated blood × appointment necessary	−0.02	0.052		
Ever donated blood × have gift	−0.012	0.053		
Ever donated blood × no gift	0.012	0.053		
Ever donated blood × opt-out	−0.622***	0.055		

Abbreviations: ASC, alternative-specific constant; MXL-I, mixed logit with interaction terms; SE, standard error; StdD, standard deviation.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Second, similar to previous research among active donors in China [12], findings from the present study indicated that small token gifts (e.g., mugs) were important to experienced and inexperienced donors alike. Small token gifts such as mugs may have limited monetary value but may effectively demonstrate gratitude to the donor. Moreover, such gifts that conspicuously acknowledge voluntary

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FIGURE 3 Scaled estimation of blood donation preference in China by donation history (MXL-I model) (n = 1185). *Higher values denote stronger preference utility. MXL-I, mixed logit with interaction terms.

donors for their voluntary blood donation may be perceived as a virtuous honour that can even enhance one's moral status and sense of virtue [16, 50]. In addition, gifts that have blood donation iconography can help evoke one's 'warm glow' feeling associated with donating, strengthen blood donor identity and confer an altruistic social status often linked to blood donation [51]. It is worth noting that the utility gained from providing a gift approximated the utility gained by reducing the blood donation volume from 400 to 200 mL.

Third, we observed that blood donation preferences for experienced and inexperienced donors were remarkably similar, except for a slight difference in preference for donation venue. Among experienced donors, blood donation stations were their favourite place to donate, but this was the least preferred venue for inexperienced donors. Inexperienced donors have never donated at a blood station and therefore may be warier of such unfamiliar venues. These inexperienced donors may prioritize safety and view hospitals as comparatively more familiar and hence more trustworthy. Experienced donors who donated at a blood donation centre may have experienced first-hand the advantages of blood donation stations over hospitals, such as shorter waiting periods [52–54].

There are several study limitations to be noted. First, the generalizability of the study results is limited because 92% of the study participants were college-educated, compared with only 19.34% of the adult national population [55]. Individuals with lower educational attainment may have different voluntary blood donation service preferences. Second, study participants were recruited from an online panel that may not reflect the preferences of individuals who are not online or not enrolled in Internet survey panels. Third, this DCE study was able to quantify blood donation preferences, but it is unclear how well such preferences can predict actual donation behaviour. Finally, the 'small gift' attribute level was accompanied by a photo of a ceramic cup featuring a heart logo. The use of different logos or photos for the small gift attribute may have elicited substantially different preference utilities for this level.

In conclusion, augmenting voluntary blood donation rates will be critical to addressing future blood shortages in China. Aligning blood donation policies with potential donor preferences may increase voluntary donation rates. Results from this nationwide DCE indicated that paid leave, small gifts and small blood donation volumes may increase donation rates in China, among inexperienced and experienced donors alike. Future studies should consider quantifying the impacts of these policies on blood donation behaviour.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Codes are available as Supporting Information.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

A tight interplay between platelet activation and mitochondrial DNA release promotes platelet storage lesion in platelet concentrates

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Abstract

Background and Objectives: Platelet storage lesion (PSL) adversely affects the quality of platelet concentrates (PCs). Platelets are prone to activation during storage. Moreover, elevated free mitochondrial DNA (mtDNA) levels in PCs are associated with a higher risk of adverse transfusion reactions. Therefore, we aimed to evaluate the correlation between platelet activation markers and mtDNA release during PC storage.

Materials and Methods: Six PCs prepared by the platelet-rich plasma method were assessed for free mtDNA copy number using quantitative real-time PCR and CD62P (P-selectin) expression by flow cytometry on days 0 (PC collection day), 3, 5 and 7 of storage. Lactate dehydrogenase (LDH) activity, pH, platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) were measured as well. The correlation between free mtDNA and other PSL parameters, and the correlation between all parameters, was determined.

Results: Significant increases in free mtDNA, MPV and PDW, and a significant decrease in platelet count and pH were observed. CD62P expression and LDH activity elevated significantly, particularly on storage days 5–7 and 0–3, respectively. Moreover, a moderate positive correlation ($r = 0.61$) was observed between free mtDNA and CD62P expression. The r values between free mtDNA and LDH, pH, platelet count, MPV and PDW were 0.81, −0.72, −0.49, 0.81 and 0.77, respectively.

Conclusion: The interplay between platelet activation and mtDNA release in promoting PSL in PCs may serve as a promising target for future research on applying additive solutions and evaluating the quality of PCs to improve transfusion and clinical outcomes.

Keywords

mitochondrial DNA, platelet activation, platelet concentrate, platelet storage lesion, transfusion reaction

Highlights

- Platelet activation and platelet mitochondrial dysfunction occur during the development of platelet storage lesion (PSL).
- We found that an interplay between platelet activation and mitochondrial DNA release from platelets promotes the development of PSL.
- Mitigating this interplay may improve the quality and safety of platelet concentrates.

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INTRODUCTION

Platelet concentrate (PC) transfusion plays a key role in the management of thrombocytopenic patients. However, during the preparation and storage of PCs, platelets undergo a number of functional and structural alterations, resulting in the development of platelet storage lesion (PSL). These changes adversely affect the shelf life, efficacy and safety of PCs, which are associated with poor transfusion and clinical outcomes [1].

During the storage of PCs, changes in platelet membrane proteins play a role in post-transfusion clearance through various mechanisms. Stored platelets are prone to activation over time, as determined by increased P-selectin (CD62P) expression on their surface [2]. CD62P expression on stored platelets is associated with fast clearance of transfused platelets from circulation by the reticuloendothelial system [3]. Therefore, the expression of CD62P on stored platelets is correlated with reduced in vivo recovery and survival upon transfusion [4, 5]. Moreover, CD62P accumulation in apheresis PCs has been shown to be involved in mediating transfusion-related acute lung injury (TRALI) [6].

PCs with increased levels of free mitochondrial DNA (mtDNA) are associated with a higher risk of adverse transfusion reactions (ATRs) [7, 8]. As a result of platelet activation during PC storage, platelet mitochondria are released into the extracellular milieu [8, 9]. The released mitochondria exist both as free organelles and encapsulated within microparticles. Mitochondria serve as endogenous substrates of bactericidal secreted phospholipase A2 IIA (sPLA2-IIA), which yields pro-inflammatory mediators, such as mtDNA after hydrolysis of mitochondrial phospholipids [10, 11]. Free mtDNA contains CpG and formylated peptides, which provoke inflammatory responses through Toll-like receptor 9 (TLR9) and formyl peptide receptors, respectively [12]. Accordingly, the elevated levels of mtDNA in blood products are associated with a higher risk of non-haemolytic transfusion reactions, acute respiratory distress syndrome and TRALI following transfusion [7, 10, 13, 14].

We have previously shown that adding resveratrol, an antioxidant polyphenol, to PCs reduced free mtDNA and maintained biochemical parameters in PCs, possibly by decreasing platelet activation [15]. Additionally, another investigation conducted by our team revealed elevated reactive oxygen species (ROS) production during the storage of PCs [16, 17]. Therefore, we thought it would be valuable to investigate the correlation between platelet activation markers and free mtDNA in PCs during the 7-day storage period.

MATERIALS AND METHODS

Preparation of PCs

Six units of PCs were randomly obtained from healthy volunteer donors who signed the consent form at the Iranian Blood Transfusion Organization (IBTO). The study was approved by the Research Ethics Committee of the High Institute for Research and Education in Transfusion Medicine under the approval code IR.TMI.REC.1398.027. PCs were prepared by the platelet-rich plasma (PRP) method from whole blood preserved with citrate phosphate dextrose adenine (CPDA1). Each platelet bag (Macopharma, France) contained 50–60 mL PCs with platelet counts above 1×10^3/μL and was stored at 22 ± 2°C in the platelet incubator with gentle agitation for 7 days. On days 0 (PC collection day), 3, 5 and 7 of storage, the study parameters were assessed.

Measuring the haematological parameters

PC samples were subjected to a calibrated cell counter (Sysmex K-1000, Kobe-Japan) on days 0, 3, 5 and 7 of storage to measure platelet count, mean platelet volume (MPV) and platelet distribution width (PDW).

Measuring the biochemical parameters

To evaluate the lactate dehydrogenase (LDH) enzyme activity of PC samples, an LDH activity assay kit (Randox, UK) based on colorimetric methods was used on days 0, 3, 5 and 7 of storage. For this purpose, 0.5 mL of samples were centrifuged at 2000*g* for 5 min and 0.2 mL of the supernatant was transferred to a Chemistry Analyzer (Hitachi 911, Japan).

Also, the pH value was assessed using a calibrated pH meter (Metrohm, Switzerland) on days 0 and 7 of PC storage.

Flow cytometric analysis of platelet activation

CD62P expression was evaluated by incubating platelets resuspended in Tyrode buffer at a density of 5×10^7/mL with FITC-labelled anti-CD62P (BD, USA) for 30 min at room temperature in the dark. To ensure the accuracy of the test, activated platelets with 0.2 unit/mL thrombin (Sigma-Aldrich) were prepared as a positive control sample. Determining possible non-specific bindings was performed by FITC-conjugated Isotype control antibody. After incubation, samples were fixed with paraformaldehyde 1% before being analysed by flow cytometry (Sysmex Partec) with the FlowMax software. Moreover, the platelet population of each PC sample was identified by labelling platelets with anti-CD41-FITC (Dako-Denmark) for 30 min. The staining of platelets for CD62P and CD41 was performed in separate tubes.

Free mtDNA quantification

Preparing PC supernatant

A two-step centrifugation protocol was performed on samples at 1600*g* for 10 min and 13,000*g* for 10 min to obtain platelet-free supernatants. The obtained supernatants were stored frozen until testing.

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DNA extraction

DNA extraction was carried out from 200 μL of PC supernatant using the QIAamp DNA Blood Mini Kit (Germany) according to the manufacturer's protocol.

Free mtDNA measurement

The quantity of free mtDNA in each PC was calculated as copy numbers by absolute quantification qPCR (Rotor-gene Q, Qiagen) with SYBR Green PCR master mix (Jena Bioscience). The quantitative real-time PCR was performed in duplicate to account for variations in sampling and software. The real-time PCR conditions included 95°C for 2 min (one cycle), 95°C for 15 s and 60°C for 35 s (40 cycles). A melting curve analysis was also performed between 70 and 94°C. Human mtDNA-specific primer sequences for the target gene NADH dehydrogenase 6 (ND6) were as follows: forward CCATCGCTGTAGTATCCAA and reverse TCGGGTGTGTTATTATTCTGA. Nuclear DNA (ncDNA) specific primers (B2MF TGTTCCTGCTGGGTAGCTCT, B2MR CCTCCATGATGCTGCTTACA) were used as the reference gene. To calculate free mtDNA copy numbers and evaluate the efficiency of the amplification, a serial dilution of the target gene was made using a mtDNA-positive control sample. To do so, hydrogen peroxide (H_2O_2) was added to a platelet suspension to induce oxidative damage. The standard curve of ND6 was subsequently prepared by serial dilutions. Real-time PCR with efficiency = 1.09, curve slope = −3.131 and $R^2 = 0.922$ was used for PC samples [18].

Testing the bacterial contamination

1 mL of sample from each PC was added to 10 mL of thioglycolate medium on days 0 and 7 of storage to test the bacterial contamination. The turbidity was checked during the 7 days of incubation at 37°C.

Statistical analysis

Values are shown as mean ± SEM. The data normality test was performed using the Shapiro–Wilk test. To study the effect of storage time on the parameters, parametric data were analysed using one-way repeated measure analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. The non-parametric data were analysed using the Friedman test followed by Dunn's multiple comparison. For pH, a paired *t*-test was performed. We assessed the correlation between free mtDNA and other PSL parameters and then between all parameters by Spearman's test using GraphPad Prism 9 software (GraphPad Prism Software, Inc., San Diego, CA, USA). An $r \leq 0.1$ is indicated as no correlation, $r > 0.1$ to <0.5 is indicated as a weak correlation, $r \geq 0.5$ to <0.8 is indicated as a good correlation and $r \geq 0.8$ is indicated as a very good correlation. *p*-Value<0.05 is considered statistically significant.

RESULTS

The negative impact of storage time on PSL parameters

To assess the effect of storage time on PSL parameters, several in vitro parameters were measured on days 0, 3, 5 and 7 of PC storage. According to the one-way repeated measure ANOVA analysis, a significant decrease in platelet count was observed throughout storage with a mean of 1177 ± 145 × 10^3/μL on the first day (Figure 1a). Additionally, the MPV value displayed a significant increase during storage, which was considerable at the end of the storage time (Figure 1b). PDW, as well as MPV, showed a significant increase for stored PCs (Figure 1c).

There was a significant increase in LDH enzyme activity during storage, which was sharper on storage days 0–3 (41.7% increase) based on Tukey's multiple comparisons analysis (Figure 1d). Also, the mean pH of stored PCs on the last day was 6.54 ± 0.15, significantly lower than day 0 (Figure 1e).

Surface CD62P expression was assessed using the flow cytometry test as a marker of platelet activation. This marker elevated significantly and reached its highest level of 43.85 ± 3.03% on day 7 of storage. Moreover, the CD62P increment on storage days 5–7 was sharper than the others (34.37% increase) (Figure 1f). Thrombin-induced platelet activation as a positive control sample gave 86.07% CD62P expression on platelets, and the mean CD41 expression on day 0 of storage was 93.57 ± 1.5% for the platelet population (Data S1).

We used SYBR green dye-based quantitative real-time PCR to determine free mtDNA copy number changes over time in PCs. According to the statistical analyses, a significant increase in free mtDNA was observed in samples during storage. The mean free mtDNA copy numbers were 2.84 × 10^7 ± 1.1 × 10^7 molecules/μL on the first day of storage. Interestingly, a rise of 190.27% (approximately a threefold increase) in mtDNA release was observed on day 3 of PC storage. However, it was not statistically significant, and only the results on days 0 and 7 of storage were statistically different (Figure 1g).

Free mtDNA correlates significantly with other PSL parameters

According to Spearman's correlations between free mtDNA and other PSL parameters (Figure 2), a good correlation ($r = 0.61$, $p = 0.001$) was observed between free mtDNA and CD62P expression as a marker of platelet activation. Also, a very good correlation with an *r* of 0.81 was analysed between free mtDNA and LDH and between free mtDNA and MPV. We observed a good correlation of free mtDNA with pH and PDW with *r* values of −0.72 and 0.77, respectively. A weak inverse relationship was also demonstrated between free mtDNA and platelet count ($r = -0.49$, $p = 0.01$).

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FIGURE 1 Changes in research parameters of platelet concentrates stored for up to 7 days. Results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (a, d, f), Friedman test (b, c, g) or paired *t*-test (e). LDH, lactate dehydrogenase; MPV, mean platelet volume; mtDNA, mitochondrial DNA; PDW, platelet distribution width; PLT, platelet count.

The correlation between all parameters

We further investigated the associations between all parameters by calculating the correlation coefficient (Figure 3). CD62P was negatively and significantly correlated with pH ($r = -0.9$, $p < 0.0001$), but had no association with platelet count ($r = -0.04$, $p = 0.86$). A good correlation was observed between CD62P and LDH ($r = 0.74$, $p < 0.0001$). CD62P was also

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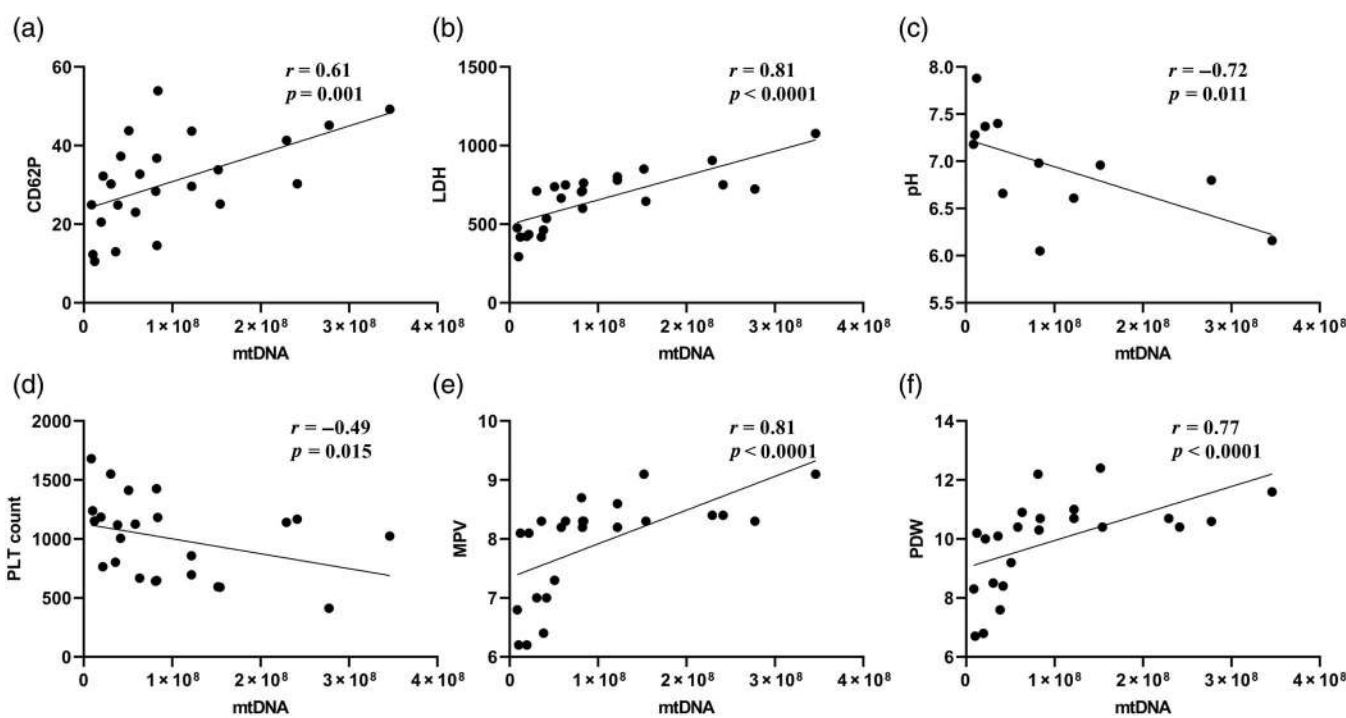

FIGURE 2 Correlation between free mtDNA and other platelet storage lesion parameters during 7 days of platelet storage. In each graph, $n = 24$, except for (c), where $n = 12$. CD62P, CD62P expression; LDH, lactate dehydrogenase enzyme activity; MPV, mean platelet volume; mtDNA, free mitochondrial DNA copy number (molecules/μL); PDW, platelet distribution width; PLT, platelet count.

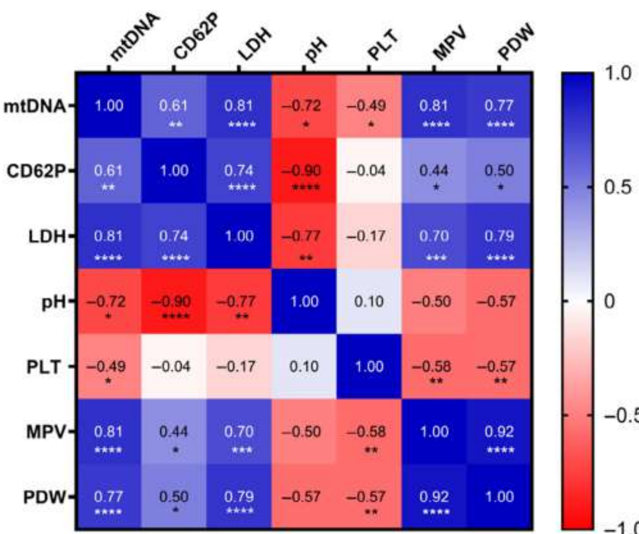

FIGURE 3 Heat map of the Spearman correlation (r) between research parameters. Red and blue squares indicate negative and positive correlations, respectively. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. CD62P, CD62P expression; LDH, lactate dehydrogenase enzyme activity; MPV, mean platelet volume; mtDNA, free mtDNA copy number (molecules/μL); PDW, platelet distribution width; PLT, platelet count.

positively correlated with MPV and PDW ($r = 0.44$, $p = 0.03$) ($r = 0.5$, $p = 0.01$).

LDH demonstrated a good positive association with MPV and PDW ($r = 0.7$, $p = 0.0001$) ($r = 0.79$, $p < 0.0001$) and an inverse correlation with pH ($r = -0.77$, $p = 0.003$). The negative correlation of LDH with platelet count was insignificant ($r = -0.17$, $p = 0.42$). The pH revealed no significant association with MPV, PDW and platelet count ($r = -0.5$, $p = 0.1$) ($r = -0.57$, $p = 0.06$) ($r = 0.1$, $p = 0.76$). Platelet count was inversely and significantly correlated with MPV and PDW ($r = -0.58$, $p = 0.003$) ($r = -0.57$, $p = 0.004$). Furthermore, MPV and PDW displayed a strong positive association with each other ($r = 0.92$, $p < 0.0001$).

Results of the bacterial culture

Bacterial screening tests were negative for all PCs.

DISCUSSION

This study aimed to investigate the correlation between free mtDNA and other PSL parameters, as well as evaluate the impact of storage time on these parameters. A significant increase in both CD62P expression and free mtDNA was observed during 7 days of PC storage. The increase in free mtDNA is consistent with our previous study on PCs stored for 7 days and the results of Chen et al. on apheresis PCs during 7 days of storage [15, 19]. However, some studies show continuous mtDNA release in the initial days of storage, followed by a decline on the last day, possibly attributed to the degradation of mtDNA during storage [8, 16].

CD62P expression and free mtDNA were positively correlated with each other ($r = 0.61$). This is consistent with a previous study demonstrating that platelet activation upon stimulation with thrombin

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leads to mtDNA release and TLR9 expression on platelets. The released mtDNA further activates platelets via TLR9 [20]. It has also been reported that the released mtDNA from activated platelets triggers further platelet activation through interaction with DC-SIGN, a platelet surface molecule [21]. Another study demonstrated a strong correlation between platelet activation, assessed by MPV/platelet count ratio, and free mtDNA using blood samples of patients during cardiopulmonary bypass ($r = 0.68$). Consequently, the study suggested that platelet activation likely contributes to inflammation following cardiopulmonary bypass by increasing mtDNA release [22]. This association can be justified by mechanisms involved in platelet activation. Multiple studies have demonstrated the dual behaviour of platelets upon activation with stimulators, such as thrombin, collagen and H_2O_2, depending on their concentrations. At low concentrations, these stimuli induce platelet activation by mitochondrial hyperpolarization, leading to increased ATP and ROS generation [23]. ROS production plays a key role in collagen-induced platelet activation through the Protein Tyrosine Phosphatase SHP2 oxidation [24, 25]. Conversely, loss of the mitochondrial membrane potential, cytochrome c release from the mitochondria, and finally platelet apoptosis are expected at high concentrations [22, 26, 27]. Hence, the regulation of platelet activation by mitochondria is not limited to supplying energy.

Our study identified a good positive correlation of LDH enzyme activity with mtDNA release and platelet activation with a significant upward trend during storage. The increase in LDH in PCs indicates platelet lysis and membrane disintegration [28]. Moreover, platelet activation and adhesion are followed by discharge of granular and cytosolic contents, such as LDH [29, 30]. Therefore, this correlation may highlight the possibility that platelet activation and subsequent platelet membrane disintegration are involved in mtDNA release into the PC milieu.

We observed a strong inverse correlation between pH and platelet activation ($r = -0.9$, $p < 0.0001$), consistent with a study that found a similar correlation in PCs ($r = -0.85$) [31]. In compliance with other investigations, we observed that CD62P expression reached its highest level at the end of the storage time with a pH value less than 6.5 [4, 31]. We should consider that the use of platelet additive solutions (PAS) in stored platelets may influence these findings due to their impact on the pH level [32]. Increased glycolysis during PC storage results in excessive lactate production, which contributes to a loss in bicarbonate buffering capacity and a drop in plasma pH [33]. The acidic milieu is able to trigger platelet activation [34]. Moreover, pH displayed a significant inverse correlation with mtDNA and LDH. As stated earlier, platelet activation is followed by increased LDH enzyme activity and mtDNA release.

Platelets undergo a morphological change from their discoid shape to spheres with pseudopods upon activation, leading to increased MPV [35]. Platelet indices, especially MPV and PDW, serve as prognostic and diagnostic markers for platelet function and activation. Increased MPV indicates a high thrombotic potential of platelets [36, 37]. In this study, we found that MPV and PDW show a strong positive correlation with each other ($r = 0.92$), but an inverse correlation with platelet count ($r = -0.58$). Moreover, we revealed that MPV and PDW are significantly correlated with CD62P, mtDNA and LDH, highlighting the association of platelet activation with mtDNA release in promoting PSL in PCs.

A recent study has demonstrated that during platelet activation, platelet mitochondria undergo a fission process to distribute evenly the energy supply throughout these large platelets [38]. As the mitochondrial fission follows the mtDNA replication [39], it may increase the mtDNA level in platelets during PSL. Moreover, the fission of platelet mitochondria contributes to a shift from oxidative metabolism to a more glycolytic metabolism [38]. A previous study has reported that upregulation of glycolysis and reduction in oxidative phosphorylation result in acidification of PCs during the 3 days of storage [40]. Therefore, the fission of mitochondria due to platelet activation might be involved in the acidic milieu of PCs during storage.

One of the limitations of this study was the small number of samples. It was not possible to obtain more samples from blood transfusion centres due to their demand for platelet products. Moreover, due to financial limitations, we could not evaluate additional parameters, including soluble P-selectin, TLR9 and sPLA2-IIA. It is also important to assess the transferability of this study to other settings where PCs are derived from buffy coat or apheresis methods, as well as the impact of different PAS.

In conclusion, we demonstrated that free mtDNA correlates with platelet activation and other PSL parameters, and this finding may shed light on the PSL mechanism. The tight interplay between platelet activation and mtDNA release in promoting the PSL in PCs may play a pivotal role in ATRs. Moreover, free mtDNA measurement could be used as a PSL marker, but it may not be a routinely measured quality control parameter due to its complexity and cost. Given the strong correlation between MPV and free mtDNA, it would be easier to use MPV as the marker for evaluating the quality of PCs. Our study suggests further research in applying novel additive solutions that can reduce mtDNA release or platelet activation to extend the shelf life, efficacy and safety of PCs and improve transfusion and clinical outcomes.

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M.R.D. supervised the experiment, K.H. performed the experiments, M.R.D., S. Samiee and S. Hajati contributed to the provision of study materials or tools, K.H. and P.B. contributed to the data collection and analysis, K.H. wrote the manuscript, M.R.D., K.H., P.B. and S. Samiee reviewed and edited the manuscript and all authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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ORIGINAL ARTICLE

Blood donation screening for hepatitis B virus core antibodies: The importance of confirmatory testing and initial implication for rare blood donor groups

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Abstract

Background and Objectives: Exclusion of blood donors with hepatitis B virus (HBV) core antibodies (anti-HBc) prevents transfusion-transmitted HBV infection but can lead to significant donor loss. As isolated anti-HBc positivity does not always indicate true past HBV infection, we have investigated the effectiveness of confirmatory anti-HBc testing and the representation of rare blood groups in anti-HBc-positive donors.

Materials and Methods: Three hundred ninety-seven HBV surface antigen-negative and anti-HBc initially reactive blood donor samples were tested by five different anti-HBc assays.

Results: Eighty percentage of samples reactive in Architect anti-HBc assay were positive by the Murex assay and anti-HBc neutralization. Eleven out of 397 samples showed discordant results in supplementary testing from the Murex confirmatory test result, and five remained undetermined following extensive serological testing. Thirty-eight percentage of anti-HBc-positive donors identified as minority ethnic

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groups compared with 11% representation in anti-HBc-negative donors ($p < 0.0001$); the frequency of the Ro blood group in anti-HBc-positive donors was 18 times higher in non-white ethnic groups.

Conclusion: Using two anti-HBc assays effectively enabled the identification of HBV-exposed and potentially infectious donors, their deferral and potential clinical follow-up. However, the exclusion of confirmed anti-HBc-positive donors will still impact the supply of rare blood such as Ro.

Keywords

anti-HBc testing, donor re-entry, ethnic groups, HBV screening, rare blood groups

Highlights

- A hepatitis B virus (HBV) core antibodies (anti-HBc) confirmatory algorithm using two different anti-HBc assays effectively delineates true and false reactivity; knowledge of false reactivity better guides deferral and follow-up strategies.
- Anti-HBc blocking and avidity assays were used to demonstrate the high efficacy of the anti-HBc confirmation strategy. These assays can be applied for confirmatory testing and to better understand the natural history of HBV infection.
- The high proportion of rare blood group donors positive for anti-HBc positive means that excluding them may impact the critical supply of rare blood. There may be a need to consider the implications of universal anti-HBc screening further.

INTRODUCTION

Around 2 billion people worldwide have serologic evidence of current or resolved hepatitis B virus (HBV) infection, indicated by the presence of antibodies to the hepatitis B core antigen (anti-HBc) [1] that typically persist lifelong following infection. It is established that HBV is transmissible by transfusion from blood donors who test negative for HBV surface antigen (HBsAg) but positive for anti-HBc [2, 3]. Many of these HBsAg-negative donors were found to have detectable HBV DNA and, therefore, recognized as having occult HBV infection (OBI) [4]. However, very low levels of HBV DNA are only intermittently detectable in donors' blood [5, 6]. Given the significant association of anti-HBc positivity with the presence of HBV DNA in the liver and the peripheral blood [7] and hence posing a risk of HBV transmission via blood transfusion, universal screening for anti-HBc has been implemented in many countries, including the United Kingdom [8]. However, transmission of HBV has been limited to anti-HBc-positive donors with low levels of antibodies against the HBV surface antigen (anti-HBs; <100 mIU/mL); hence, donors with higher anti-HBs levels can potentially be considered safe [9].

Identification of HBsAg-negative donors with potential long-term persistent HBV infections is further complicated by the limited specificity of anti-HBc assays used for screening, with up to 60% of initially reactive samples remaining unconfirmed [10–13]. The existence of an immunoglobulin M component in commercial anti-HBc total assays inherently restricts specificity further [14]. Specificity can be improved using robust confirmatory testing, including secondary anti-HBc assays or neutralization tests that measure the blocking of anti-HBc reactivity by using a soluble recombinant HBV core antigen (rHBcAg) [15]. Other means to identify if borderline anti-HBc positivity is a result of non-specificity or low levels of circulating anti-HBc [16] include the measurement of anti-HBc avidity, where the strength of binding of anti-HBc antibodies is measured by an avidity index (AI), which increases between acute and chronic HBV infections [17].

There is particular concern that the exclusion and deferral of anti-HBc-positive donors could negatively impact donor populations with rare blood groups associated with the absence of high-incidence red cell antigens. Indeed, it has been shown that most donors with OBI in the United Kingdom were born in areas of the world where HBV infection is endemic [18]. This is also where 55% of donors of Black ethnicities have the Ro blood subtype, a subtype only seen in 2% of all UK donors [19]. Rare blood phenotypes are in demand. The Ro subtype is frequently transfused to Ro patients with sickle cell disorder [19] to prevent stroke and crisis. Furthermore, the rare Rh type, rr, denotes a rare subtype of ABO group O Rh D negative, which is frequently transfused in emergencies [20]. The full impact of excluding anti-HBc-positive donors on the supply of units with rare blood groups in the United Kingdom remains unknown.

In this study, we have first investigated the effectiveness of two different anti-HBc assays to determine true anti-HBc positivity by applying an anti-HBc blocking assay and anti-HBc avidity test in a cohort of 397 blood donors reactive in one anti-HBc assay. We have also investigated the representation of rare blood groups in deferred anti-HBc-positive donors with high levels of anti-HBs antibodies.

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MATERIALS AND METHODS

Clinical specimens

Three hundred ninety-seven ethylenediaminetetraacetic acid (EDTA) plasma samples were obtained from NHS Blood and Transplant (NHSBT) that had tested negative for HBsAg (Abbott Prism or Alinity S) and repeatedly reactive for anti-HBc (Architect, Abbott total anti-HBc II assay; signal to cut-off [S/CO] ratio ≥1.0 being considered reactive) between 16 June 2022 and 13 January 2023. These samples had also been screened negative for HBV DNA in pools of 24 (Roche Cobas 6800; 95% limit of detection [LOD] 1.4 IU/mL; calculated 95% LOD 33.6 IU/mL in individual donor level when tested in pools of 24) and for anti-HBs (Abbott Architect). Samples with anti-HBs levels <100 mIU/mL were tested further at NHSBT using a second anti-HBc assay (Murex total anti-HBc, DiaSorin) in duplicate, for antibodies against the HBV e antigen (anti-HBe; Biomerieux Vidas) and for HBV DNA using individual nucleic acid testing (NAT; Grifols Procleix Panther; 95% LOD 4.5 IU/mL). Anti-HBc positivity was defined as anti-HBc positive in the Architect and Murex assays and either anti-HBe or anti-HBs positive. In contrast, samples that were only anti-HBc positive in Architect and Murex but negative for other HBV markers were considered anti-HBc inconclusive. Architect positive but Murex negative were considered anti-HBc negative.

Clinical HBV testing at NHSBT was limited to samples with anti-HBs titres <100 mIU/mL as described above but not on samples with higher anti-HBs titres. To harmonize testing for the entire sample set regardless of anti-HBs titre, we tested samples with anti-HBs titres >100 mIU/mL for HBV DNA individually (Roche Cobas 6800; 95% LOD 1.4 IU/mL; $n = 250$). We then assayed all 397 samples by anti-HBc (Murex total anti-HBc, DiaSorin, Italy) and anti-HBe (FineTest Human HBeAb [hepatitis B virus E Antibody] ELISA kit, Wuhan, China) testing following manufacturers' instructions. Thirteen random anti-HBs <100 mIU/mL samples that were initially tested with the Murex assay at the clinical laboratory were also tested at the research laboratory, where a correlation of similar anti-HBc S/CO ratios with was found ($r = 0.940$, $p < 0.001$). Furthermore, testing of 19 random anti-HBs <100 mIU/mL samples (consisting of 10 anti-HBe negatives and 9 anti-HBe positives) with the FineTest kit revealed 100% concordant qualitative results with the VIDAS assay, and quantitative values strongly correlated ($r = 0.794$, $p < 0.001$). A further 26 HBV DNA-positive samples were used as additional positive controls in this study [21].

Demographic data were obtained from donors at the time of donation. The self-reported ethnicities of all 297,949 blood donors screened for anti-HBc in the 1 year since anti-HBc screening began in England from 30 June 2022 to 30 June 2023 were collated for comparison. The ethics statement is detailed in the supplementary material.

Assay development

In-house non-blocking and blocking assays were developed to study anti-HBc immune responses. For blocking anti-HBc reactivity using rHBcAg, 50 μL of each antigen dilution was pre-incubated with 50 μL of plasma dilution before plate washing and subsequent addition of immunoglobulin G (IgG). If specific anti-HBc IgG antibodies were present, these would be neutralized by rHBcAg. Assay development included standard curve calibration, negative run control and applying the blocking principle to the Murex blocking assay (Figures S1 and S2). Samples were diluted according to the screening Architect S/CO ratios aiming for OD values between 1.0 and 2.0 to normalize the denominator for calculating blocking percentages. An in-house avidity assay was also developed (Figures S3 and S4).

Rare donor analysis

Donors with rare blood types, alongside their ethnicities, were identified from the first anonymised 981 blood donors who were anti-HBc repeat reactive with anti-HBs ≥100 mIU/mL in the first 6 months of anti-HBc screening in England between 31 May 2022 and 30 November 2022. In the context of this study, rare blood types were defined as the following Rh genotypes: DcE/DcE (R2R2), Dce/dce (Ror, shortened to 'Ro') and dce/dce (rr). The number of positive anti-HBc and high titre anti-HBs donors of different ethnic groups within groups of rare Rh blood types was compared with the number of all active donors in the same ethnic groups from 30 June 2022 to 30 June 2023, excluding the 981 anti-HBc-positive donors.

Statistical analyses

Data normality was assessed with Shapiro–Wilks tests, where normal data are displayed as mean ± SD and non-normal data as median [interquartile range] where applicable. Spearman's correlation compared assay results between research and diagnostic laboratories. Fisher's exact tests compared categorical variables. Mann–Whitney *U* or *t*-tests compared continuous variables between two groups, whereas the Kruskal–Willis test or one-way analysis of variance (ANOVA) compared continuous variables with more than two groups. Receiver operating characteristic (ROC) analysis investigated the sensitivities and specificities of anti-HBc Architect ratios and anti-HBe titres that may predict anti-HBc positivity. All analyses were performed with GraphPad Prism (v10.0.2, LLC). Statistical significance was set at $p \leq 0.05$.

RESULTS

Validation of anti-HBc blocking assay

Validation of the assay was performed using 147 samples of known anti-HBc-positive status (all reactive in two anti-HBc assays [Abbot Architect and Murex] and one anti-HBe assay [Murex or Finetest]) and 55 anti-HBc-negative controls (weakly reactive in one anti-HBc assay [Abbott Architect] but negative in further anti-HBc assay

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[Murex] and for anti-HBs as well as for anti-HBe), selected from 397 study samples. In addition, 26 previously characterized HBV DNA-positive samples were included as further positive controls [21]. Architect anti-HBc ratios correlated better with in-house IU/mL values than Murex anti-HBc ratios (Figure S5).

All anti-HBe-positive and HBV DNA-positive control samples were blocked by soluble rHBcAg in the HBcAg ELISA based on a greater than 50% reduction of anti-HBc binding (range 69%–94%; $n = 173$; Figure 1a). Architect, Murex and in-house anti-HBc titres were significantly higher ($p < 0.0001$) in the positive than in the negative controls (Figure 1b). However, two anti-HBc-negative controls were also blocked, with anti-HBc titres higher than the third quartile above the negative control mean (Figure 1). The remaining samples reactive in the Architect anti-HBc assay ($n = 195$) were then tested for blocking. Combining the results, significantly higher anti-HBc titres in Murex, Architect and rHBcAg ELISA assays were observed in blocked samples than unblocked or unreactive in the rHBcAg ELISA (Figure 1c; $n = 397$).

Serological correlates of confirmed anti-HBc positivity

A total of 317 of 397 anti-HBc initial Architect-reactive samples were positive in the Murex assay (80%). Of these, reactivity in the rHBcAg ELISA was blocked by soluble rHBcAg in 308 samples (308/317; 97%). However, reactivity was also blocked in two Architect-positive, Murex-negative, non-confirmed, presumed anti-HBc-negative samples (2/80, 2,5%; Figure 2). The neutralization observed in the latter two samples could not be blocked using a cell lysate from the strain DH5α of *Escherichia coli* with a comparable GCE33-YFP expression plasmid without rHBcAg, ruling out non-specificity from that source.

Anti-HBc avidity

Ninety-nine samples positive in the in-house anti-HBc assay were tested to investigate the relationship between anti-HBc avidity (AI) and anti-HBc reactivity. Reactivity in anti-HBc assays was highly predictive of avidity (Figure 3): anti-HBc ratios of ≥4.75 and ≥6 in the Architect and Murex assays, respectively, predicted AI values of >0.4 and excluded lower AI and anti-HBc titres ≥14 IU/mL in the in-house assay predicted AI values of >0.5 and excluded lower AI. When assessing the two discrepant Architect-positive but Murex-negative samples blocked in the in-house blocking assay, both had similarly low avidities irrespective of positivity in the Monalisa assay.

Overall, five (1%) anti-HBc initially Architect-reactive samples remained anti-HBc indeterminate after extensive confirmatory testing (Figure 2; Table 1). Seventy-nine percentage of anti-HBc initial Architect-reactive samples were genuine anti-HBc positive, whereas 20% were confirmed to be false positive. Ninety-eight (166/170) of isolated anti-HBc-positive samples concurrently Architect and Murex positive without anti-HBe antibodies were shown to be genuinely anti-HBc positive.

HBV DNA detection by individual NAT

Individual NAT identified one HBsAg-negative donor with anti-HBc antibodies positive for HBV DNA (Ct 36.4; IU/mL unavailable) with anti-HBs >100 mIU/mL from the 397 tested. This donor was known to have recently received an HBV vaccine booster.

Characterization of anti-HBc reactivities

Comparisons of initial anti-HBc screening ratios between anti-HBc confirmed positives, indeterminates and false positives revealed significant differences between groups in anti-HBc reactivity ($p < 0.0001$; Figure 4). There were significantly higher S/CO ratios for anti-HBc true positives (6.3 [4.0–7.4]) compared with anti-HBc indeterminates (1.5 [1.2–2.1]; $p < 0.0001$) and anti-HBc false positives (1.5 [1.1–1.9]; $p < 0.0001$) by post hoc Dunn test, whereas anti-HBc ratios between anti-HBc indeterminates and false positives were similar ($p > 0.999$). Comparisons of anti-HBe status in anti-HBc confirmed that positive donors showed significantly higher anti-HBc ratios in anti-HBe-positive than anti-HBe-negative donors (7.3 [6.3–7.6] vs. 4.6 [2.6–6.5], respectively; $p < 0.0001$).

Predictors of anti-HBc true positivity

The value of anti-HBc reactivity in the Architect screening testing and the presence of anti-HBe to predict true and false anti-HBc positivity were determined quantitatively by ROC analysis using anti-HBc Architect and anti-HBe sample-to-control ratios. An Architect ratio of 2.4 showed 87% sensitivity and 85% specificity in predicting anti-HBc true positivity (Figure 5), whereas an anti-HBe cut-off of 0.6 would have 86% sensitivity and 90% specificity.

Ethnic group comparisons

Sixty-one percentage of the 397 HBsAg-negative but anti-HBc repeat reactive donors were male, and 39% were female. Donors' median age was 47 years [36–59 years]. Fifty-four percentage described themselves as White British/Irish ethnicity, 13% as other White ethnicities, five donors did not disclose their ethnicities and the remaining 31% as other ethnic groups (specific ethnic groups found in Table S1). The proportion of minority ethnic groups in anti-HBc true-positive donors was significantly higher than their representation among all blood donors (38% vs. 11%; $p < 0.0001$; Figure 4). The proportions of 'Other Ethnic Groups' (38%) and 'Other White Background' (17%) donors in anti-HBc true-positive donors were also significantly higher than in anti-HBc indeterminate (11% minority ethnic groups and 0% Other White Background; $p < 0.0001$), respectively, and anti-HBc false-positive donors (7% minority ethnic groups and 3% Other White Background; $p < 0.0001$). Indeterminate and false-positive groups showed similar ethnic backgrounds ($p = 0.686$), and both groups showed similar proportions of minority groups to those of all blood donors ($p = 0.733$ and $p = 0.842$, respectively).

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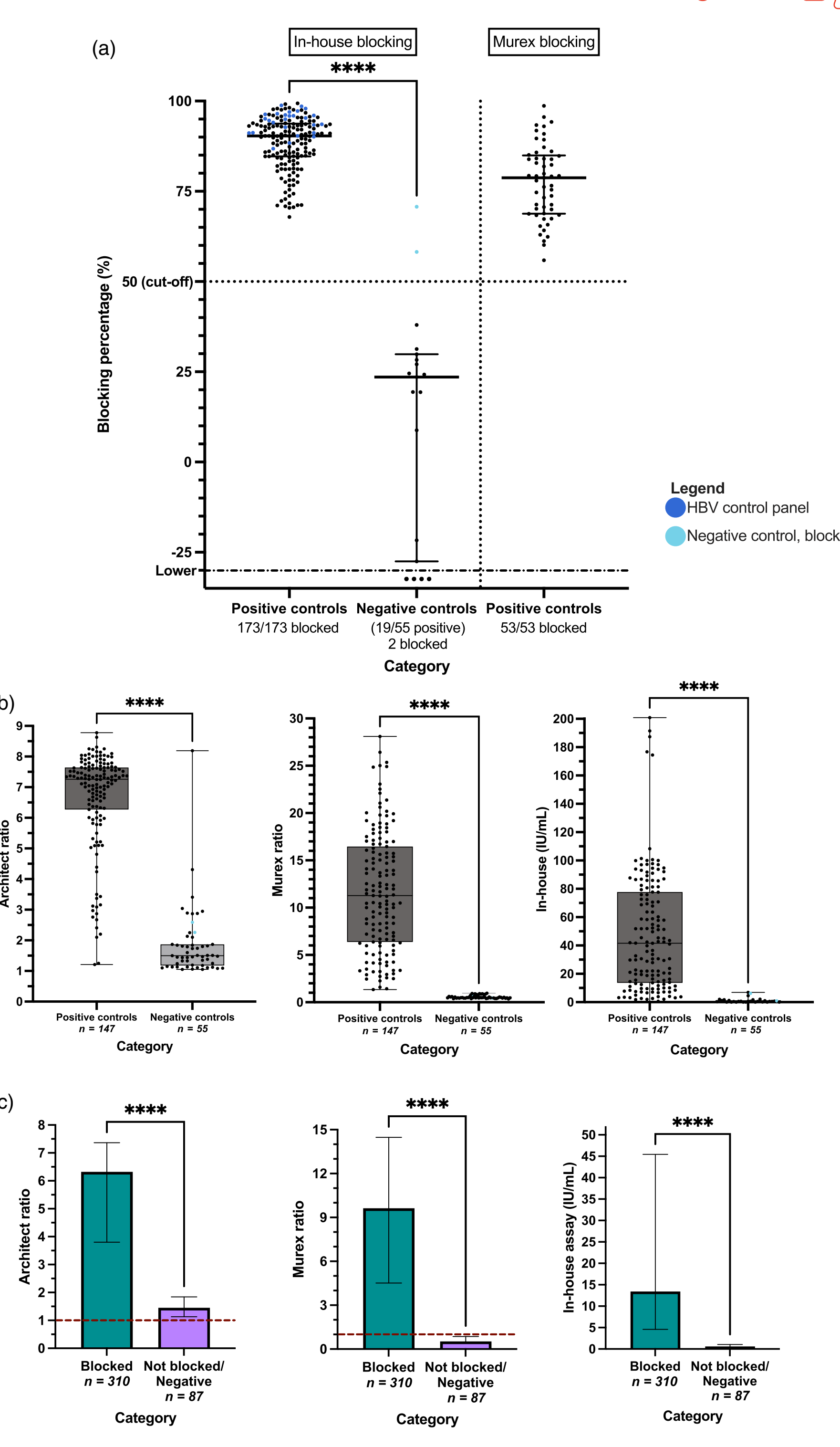

FIGURE 1 (a) Median [interquartile range] blocking percentages in all 173 hepatitis B virus (HBV) core antibody (anti-HBc)-positive controls (147 anti-HBc reactive in both screening assays, anti-HBe positive and 26 samples from the HBV control panel) and anti-HBc-negative controls (anti-HBc negative in the second screening assay, antibodies against the HBV e antigen (anti-HBe) negative and antibodies against the HBV surface antigen (anti-HBs) negative) tested in the in-house assay. Thirty-two of 51 negative controls were anti-HBc negative in the in-house assay, and the other 19 controls underwent blocking. The blocking percentages of 23 anti-HBc-positive controls tested in the Murex assay are also shown; (b) comparison of Architect, Murex and in-house anti-HBc ratios between positive and negative controls, showing all quartiles; (c) comparisons of the median [interquartile range] Architect, Murex and in-house anti-HBc ratios between donor samples that were anti-HBc blocked ($n = 310$) and samples that were either not blocked or were anti-HBc negative ($n = 87$). The dotted lines represent the 1.0 cut-off value for the anti-HBc screening assays. ****$p < 0.0001$ from Mann–Whitney U tests.

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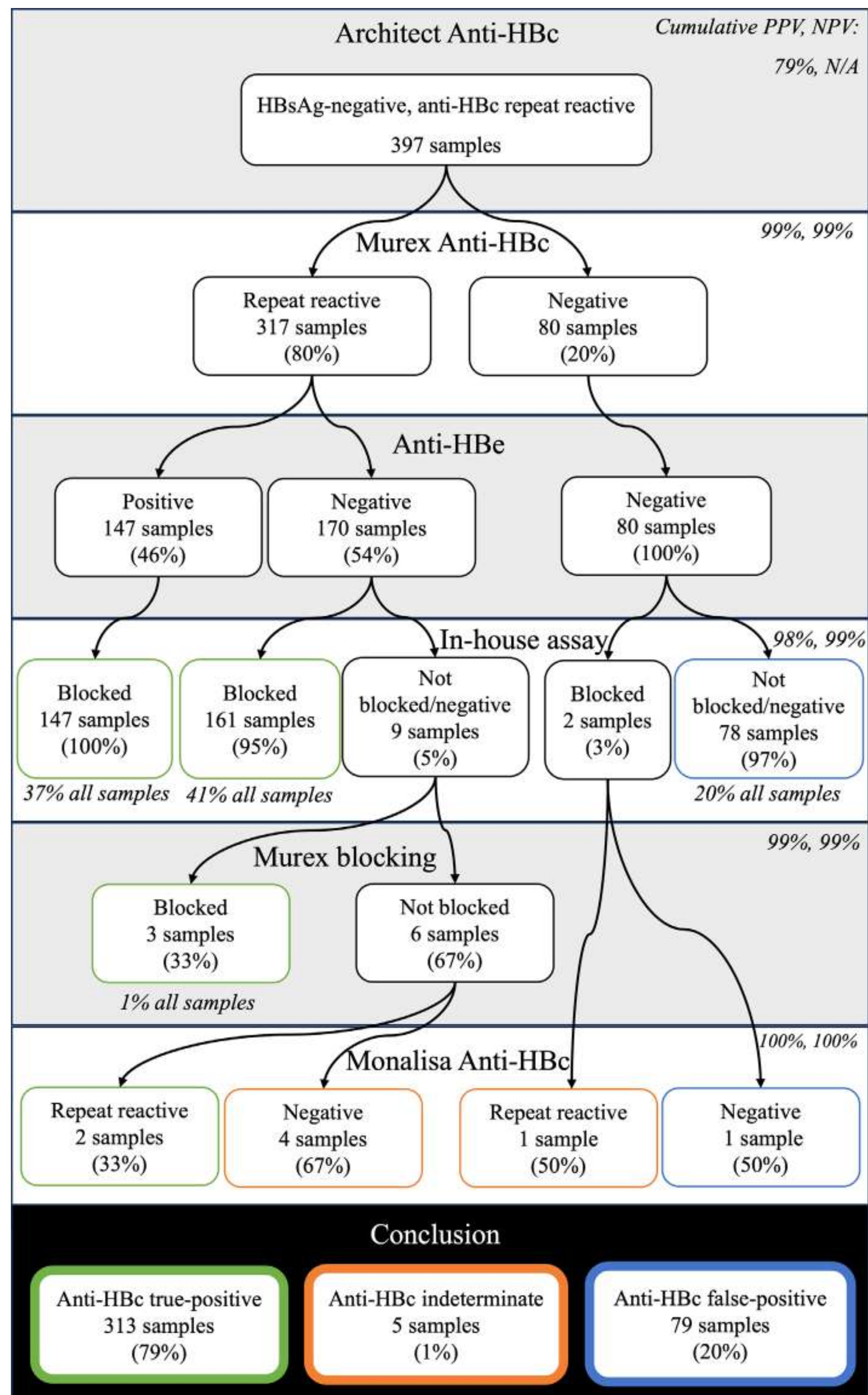

FIGURE 2 The flow diagram shows the delineation of hepatitis B virus (HBV) core antibody (anti-HBc) reactivity based on extensive testing of the 397 HBV surface antigen (HBsAg)-negative and anti-HBc initial reactive samples on the Architect assay. Testing included the Murex anti-HBc assay, anti-e antibody (anti-HBe) testing, in-house anti-HBc blocking assay, blocking on the Murex assay and the anti-HBc LIASION test. The cumulative positive predictive value (PPV) and negative predictive value (NPV) of the five anti-HBc assays to the true-positive and false-positive samples in the 'Conclusion' are shown in the top-right of each anti-HBc assay, where minimal changes are observed after the Murex assay. N/A: NPV not available since this was the first test performed in the algorithm.

Rare blood groups

Frequencies of selected blood groups were determined in the 981 anti-HBc-positive donors with anti-HBs ≥100 mIU/mL identified in England's first 6 months of anti-HBc screening. Of these, 301 (31%) had a rare blood type. There was a significantly higher proportion of rare blood group donors of White Other and 'Other Ethnic Groups' in anti-HBc-positive donors than in negative donors (29% vs. 7%,

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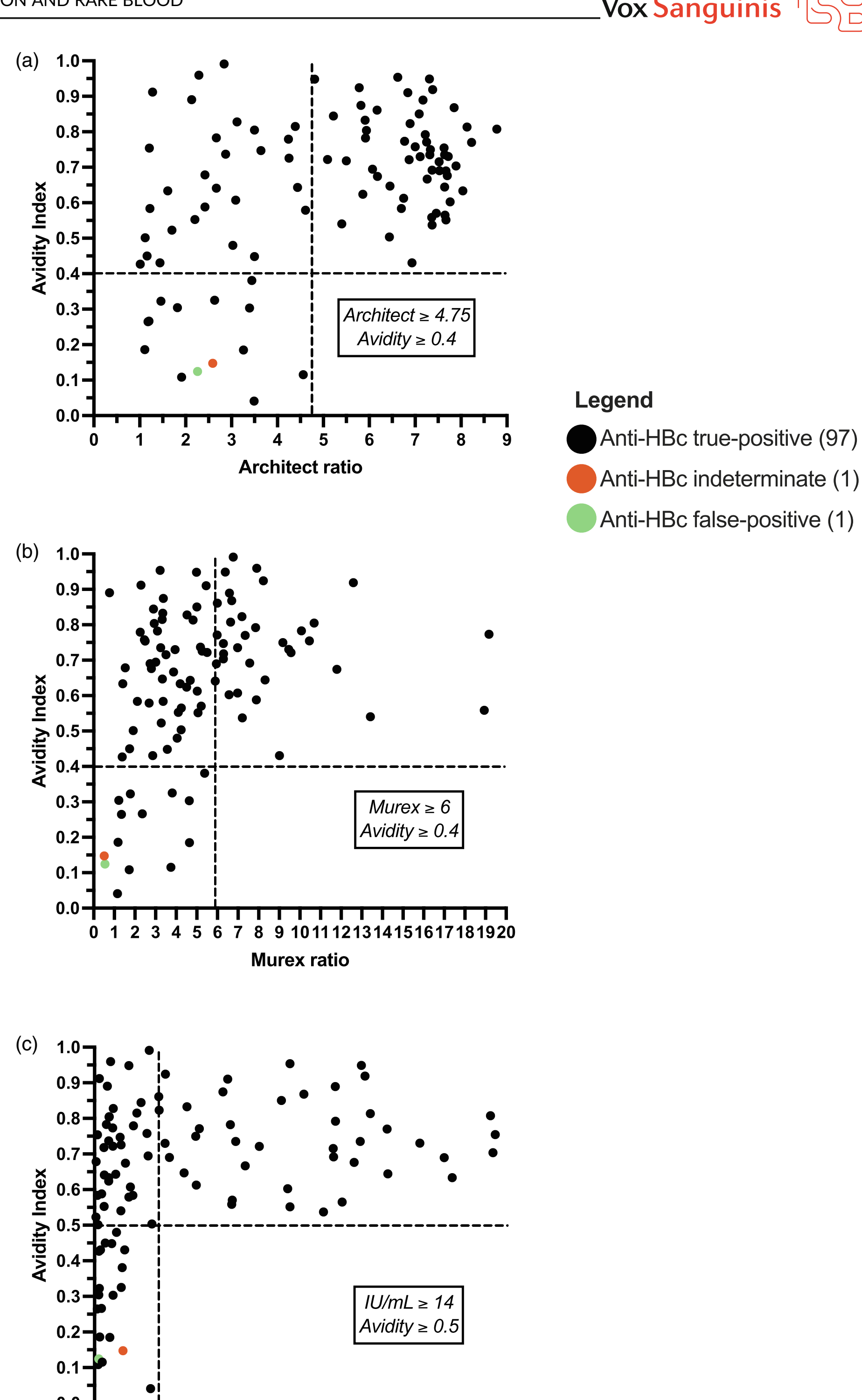

FIGURE 3 Correlation between avidity and hepatitis B virus core antibody (anti-HBc). (a) Architect signal to cut-off (S/CO) ratio ($r^2 = 0.196$), (b) anti-HBc Murex S/CO ratio ($r^2 = 0.109$) and (c) anti-HBc IU/mL from in-house assay ($r^2 = 0.006$), for all 99 samples tested for avidity and blocked. The dashed lines separate each anti-HBc ratio into a cut-off value for samples with lower avidity.

$p < 0.0001$; and 31% vs. 26%, $p = 0.033$; Table 2). Although there were similar proportions of R2R2 donors across ethnic groups ($p = 0.819$), there were significantly more Ro donors in anti-HBc-positive donors than anti-HBc-negative donors (8% vs. 3%, $p < 0.0001$), where the proportion of 'Other Ethnic Groups' Ro donors was 19 times that of White British/Irish donors. There was also a significantly higher proportion of rr donors of White Other ethnicities in anti-HBc-positive than all donors (25% vs. 2%, $p < 0.0001$).

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TABLE 1 Serological and demographic characteristics of the 11 inconclusive samples identified from the in-house blocking assay.

Sample	Architect (ratio)	Murex (ratio)	In-house blocking (%)	Murex blocking (%)	Monalisa (ratio)	Anti-HBe (ratio)	Anti-HBs (ratio)[a]	Avidity index	Ethnicity
Pos-1	1.15	**5.94**	NA	**73**	**1.132**	0.55	>1000	NA	White British/Irish
Pos-2	1.20	**2.35**	24	**68**	**2.256**	0.55	>1000	0.266	Other Ethnic Groups
Pos-3	1.72	**3.23**	NA	**87**	**1.178**	0.62	0.00	NA	White British/Irish
Pos-4	1.70	**3.28**	−44	6	**1.938**	0.52	462	0.523	White British/Irish
Pos-5	2.42	**1.53**	21	38	**2.45**	0.39	2.18	0.678	White British/Irish
Ind-1	1.29	**2.24**	NA	16	0.709	0.00	0.29	NA	White British/Irish
Ind-2	1.32	**1.91**	NA	47	0.946	0.00	1.52	NA	White British/Irish
Ind-3	1.53	**1.23**	NA	11	0.686	0.46	0.00	NA	White British/Irish
Ind-4	3.61	**1.17**	NA	9	0.945	0.56	0.36	NA	White British/Irish
Neg-1	2.26	0.549	**58**	NA	0.532	0.41	1.06	0.124	White British/Irish
Ind-5	2.59	0.512	**71**	NA	**3.441**	0.41	2.30	0.148	White British/Irish
Median [IQR] values for all anti-HBc positives and negatives identified from in-house anti-HBc blocking assay									
Positives ($n = 308$)	6.34 [3.88–7.37]	9.68 [4.64–14.50]	87 [81–93]	79 [69–85]	-	0.95 [0.62–3.03]	471 [95–2000]	0.710 [0.566–0.805]	-
Negatives ($n = 78$)	1.45 [1.13–1.84]	0.502 [0.443–0.735]	9 [−23–24]	NA	-	0.43 [0.40–0.53]	1 [0–53]	NA	-

Note: Samples are labelled according to their final status: Murex blocking assay +/− Monalisa assay (Pos, Positive), not blocked on the Murex and negative on Monalisa (Ind, Indeterminate), Murex negative and positivity on Monalisa only (Ind, Indeterminate) and Murex negative and Monalisa positive (Neg, Negative). Bold values indicate positive values according to the assay protocols. Average ratios are shown in the table. NA indicates where the blocking and avidity assays were not done since anti-HBc was negative on the assay.

Abbreviations: anti-HBc, hepatitis B virus core antibodies; anti-HBe, hepatitis B virus e antibodies; anti-HBs, hepatitis B virus surface antibodies; IQR, interquartile range.

[a]Where anti-HBs titres were >1000 IU/L, 2000 IU/L was used as an approximation to obtain average values.

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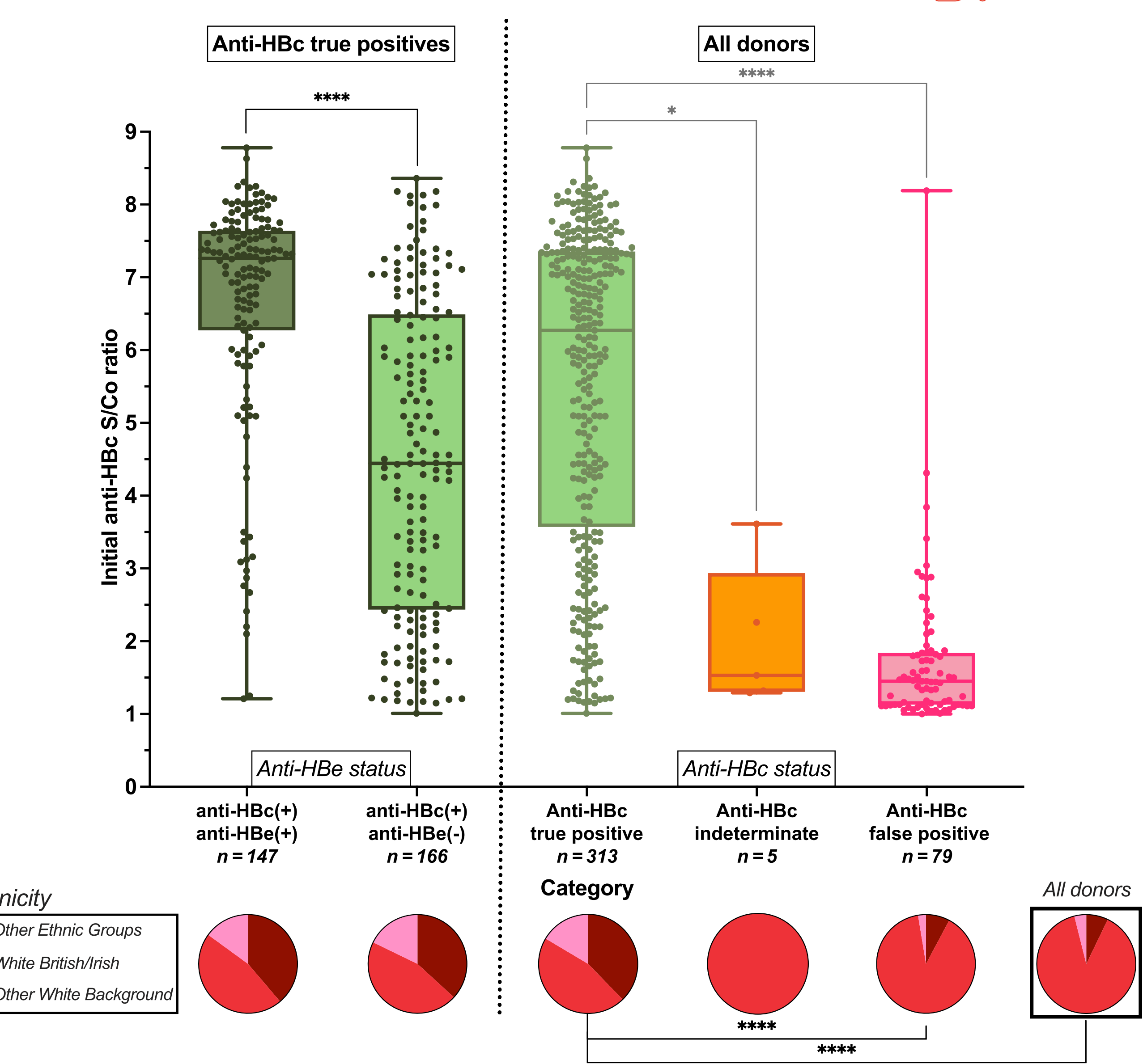

FIGURE 4 Comparisons of median [interquartile range] initial hepatitis B virus core antibody (anti-HBc) signal to cut-off (S/CO) ratios between the 397 donor samples categorized by anti-HBc genuine positivity, false positivity and indeterminate following the extensive testing detailed in Figure 2. The true-positive samples are further classified by HBV anti-e antibody (anti-HBe) status on the left hand of the graph, separated by a dotted line. The proportion of minority ethnic group donors for each category of donors is indicated, as well as from all donors in 2021. The Mann–Whitney *U* test compared anti-HBe status (darker coloured line), whereas the Kruskal–Willis test with post hoc Dunn's compared between the positivity categories (light coloured lines) and Chi-squared tests compared ethnic groups, where ***$p < 0.001$ and ****$p < 0.0001$.

DISCUSSION

This study investigated the specificity and effectiveness of current screening and confirmatory testing of donors for anti-HBc antibodies. The association between anti-HBc blocking and anti-HBc confirmation in the Murex assay demonstrated the latter's efficacy in delineating true and false anti-HBc-positive donors. Compared with the 80% specificity of the Architect assay, confirmation in the Murex assay reduced false positivity to around 1% of initially reactive donors (4/317) and 1% false negativity (1/80). Confirmation of anti-HBc reactivity showed a positive predictive value of 99% on Architect anti-HBc-positive samples. Determination of the true seroprevalence of anti-HBc positivity in donors enabled the investigation of the effects of anti-HBc-positive donor exclusion on the blood supply.

Specificity of anti-HBc testing is important and will help in communicating to donors if they have had a past HBV infection and providing appropriate advice about their future risk of developing HBV-associated progressive liver disease. HBV infection still carries a stigma, and many individuals deny having had a previous HBV infection, especially when the source of infection might simply relate to

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Sensitivity (%)	Specificity (%)	Threshold anti-HBc S/Co ratio
95	53	1.48
90	80	2.10
87	85	2.36
82	90	2.88
77	95	3.40
2	100	8.21

Sensitivity (%)	Specificity (%)	Threshold anti-HBe S/Co ratio
95	68	0.47
90	80	0.55
86	90	0.57
80	95	0.60
54	100	0.83

FIGURE 5 Receiver operating characteristic analysis to investigate Architect signal to cut-off (S/CO) ratio (green) and HBV anti-e antibody (anti-HBe) ratio (purple) thresholds that may predict HBV anti-core antibody (anti-HBc) true positivity. For this analysis, anti-HBc true positives and true negatives (false positives) were utilized from this study's 397 samples, excluding the five indeterminate samples previously shown in Figure 2. The 90%/95% sensitivity and specificity threshold values for anti-HBc and anti-HBe are shown in the tables below the plot and 100% specificity thresholds for anti-HBc positivity.

them being born in HBV high-endemicity areas. Our application of blocking and avidity assays showed that positivity in two anti-HBc assays strongly predicted true anti-HBc-positive status, irrespective of assay S/CO ratios or anti-HBe/anti-HBs status. These results are highly relevant in interpreting isolated anti-HBc reactivity in donors or patients in general. Supplementary assays such as anti-HBc blocking may differentiate between past HBV infection and false reactivity in HBV-naïve donors [22] and provide an IgG-specific measurement that may increase specificity compared with assays measuring total anti-HBc. Testing algorithms using two different anti-HBc assays would not only reassure blood donors but would also help clinicians who need to balance their decisions on starting antiviral prophylaxis based on isolated, non-confirmed anti-HBc results only, as recommended by the European Association for the Study of the Liver (EASL) [23]. This testing algorithm may also be validated in other settings with alternative anti-HBc assays to provide equivalent confirmation of anti-HBc reactivity. Another strategy to identify false reactive donors would be utilizing a threshold value [13]. We found that below a cut-off value of 2.36, the Architect anti-HBc II assay showed high sensitivity and specificity in predicting anti-HBc true reactivity. Using a threshold value is supported by our finding that samples with higher anti-HBc titres showed consistently higher high functional affinity (avidity); the demonstration of maturity of anti-HBc antibodies is consistent with genuine past HBV infection.

The rate of anti-HBc false reactivity of 20% on the Architect anti-HBc II assay, found in this study following extensive testing, is much

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TABLE 2 Comparisons of the proportion of ethnic groups between anti-HBc-positive donors ($n = 981$) and anti-HBc-negative donors ($n = 794{,}861$) for all rare blood types and each Rh blood type: R2R2, Ro and rr.

	Ethnic group			
	White British/Irish, *n* (%)	White other, *n* (%)	Other ethnic groups, *n* (%)	All groups, *n* (%)
All rare blood types				
Anti-HBc pos	119 (31%)	67 (29%)	115 (31%)	301 (31%)
Anti-HBc neg	200,019 (29%)	2666 (7%)	18,436 (26%)	221,121 (28%)
p-value	0.431	<0.0001	0.033	0.050
R2R2				
Anti-HBc pos	8 (2%)	6 (3%)	6 (2%)	20 (2%)
Anti-HBc neg	13,805 (2%)	779 (3%)	1159 (2%)	15,743 (2%)
p-value	0.855	0.46	>0.999	0.819
Ro				
Anti-HBc pos	3 (1%)	3 (1%)	82 (19%)	88 (8%)
Anti-HBc neg	15,372 (2%)	971 (2%)	10,494 (15%)	26,837 (3%)
p-value	0.055	0.385	0.014	<0.0001
rr				
Anti-HBc pos	108 (28%)	58 (25%)	27 (7%)	193 (20%)
Anti-HBc neg	170,842 (25%)	916 (2%)	6783 (10%)	178,541 (22%)
p-value	0.14	<0.0001	0.156	0.039

Note: The number of donors in each ethnic group for each blood type is shown, as well as the proportion of those donors representing all donors in that ethnic group for anti-HBc positive or negative donors. Fisher's exact tests were used to compute *p*-values.
Abbreviation: anti-HBc, hepatitis B virus core antibodies.

higher than the 6% rate in a previous study in a smaller population of 79 initially reactive samples [24]. Our rate is comparable to a German study of 22% using a confirmatory strategy of two older anti-HBc platforms [12], suggesting minimal changes in the false positivity rate of anti-HBc screening assays over time despite claimed improvements.

Our findings also show that all anti-HBe positivity was completely concordant with confirmed anti-HBc positivity and that anti-HBe positivity can, therefore, be utilized as a specific marker for past HBV infection [11] but not as a marker for OBI since most OBI cases are anti-HBe negative [21]. However, we suggest that the 0.6 to 1.0 range of anti-HBe results may constitute a zone of uncertainty: an anti-HBe S/CO threshold of 0.57 had high sensitivity and specificity in predicting anti-HBc true reactivity, and all donations with high 'negative' anti-HBe ratios above 0.83 were genuine anti-HBc positives. Furthermore, negativity in the anti-HBe assay has less predictive value; based on anti-HBc confirmatory testing, we found that the sensitivity of anti-HBe as a biomarker to identify resolved infection was limited. This may result from an increased prevalence of HBeAg-deficient HBV variants [13], the natural disappearance of detectable anti-HBe antibodies following recovery from infection and the possibility that anti-HBe antibodies may never develop during the natural history of HBV infection [25]. There is a need for alternative biomarkers; we have plans to investigate the potential use of new biomarkers, such as HBV core-related antigen (HBcrAg) [23, 26], for serological testing.

A further question is whether deferring anti-HBc-positive donors significantly impacts the supply of units with rare blood groups. We found that 38% of anti-HBc-confirmed-positive donors (in the absence or presence of other HBV markers) comprised minority ethnic groups, consistent with previous information from donors originating from countries with endemic patterns of HBV infection [27]. These donors are more likely to be Rh type D positive [28]; we additionally found that rare Rh phenotypes were more common in anti-HBc-positive donors from minority ethnic groups, whereas the proportion of rare groups in White British/Irish anti-HBc-positive donors was comparable to HBV-unexposed donors. The extensive minority ethnic group background of deferred anti-HBc-positive donors with high anti-HBs levels (≥100 mIU/mL) is more likely to have the rare Rh phenotypes Ro and rr that are critically needed for patients with sickle cell disorder to lower the risk of alloimmunisation.

The potential acceptance of anti-HBc-positive blood donors with high anti-HBs titres has been previously considered a safe means to maintain an adequate blood supply with sufficient rare blood types. However, we found one anti-HBc-positive donor with high anti-HBs levels (vaccine-boosted) of 'Other Ethnic Groups' to have detectable viraemia out of 397 donors screened for HBV DNA, although whether infectivity in this donation would have been neutralized by high levels of anti-HBs remains unknown. It is important to note that HBV transmission from anti-HBc-positive donors with high anti-HBs levels is rare and hence assumed to be a very low risk [9].

In conclusion, the development and application of anti-HBc blocking and avidity assays combined with testing for anti-HBe demonstrated relatively high specificity and sensitivity of the Murex anti-HBc assay for confirming donors reactive from screening with the

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Abbott Architect assay. Indeed, a relatively simple confirmatory algorithm with two anti-HBc assays would almost eliminate false-positive results. The impact of anti-HBc screening and excluding all positives to rare blood group types needs further consideration.

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M.X.F., J.I., C.R. and V.N. performed the research and collected the data; M.X.F. wrote the first draft of the manuscript; H.H. and P.S. designed the research study and critically edited the manuscript; all authors analysed the data and reviewed the manuscript; H.H., M.A. and P.S. supervised the research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original datasets used and analysed in this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Blood lactate after pre-hospital blood transfusion for major trauma by helicopter emergency medical services

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Abstract

Background and Objectives: The appropriate use of blood components is essential for ethical use of a precious, donated product. The aim of this study was to report in-hospital red blood cell (RBC) transfusion after pre-hospital transfusion by helicopter emergency medical service paramedics. A secondary aim was to assess the potential for venous blood lactate to predict ongoing transfusion.

Materials and Methods: All patients who received RBC in air ambulance were transported to a single adult major trauma centre, had venous blood lactate measured on arrival and did not die before ability to transfuse RBC were included. The association of venous blood lactate with ongoing RBC transfusion was assessed using multivariable logistic regression analysis and reported using adjusted odds ratios (aOR). The discriminative ability of venous blood lactate was assessed using area under receiver operating characteristics curve (AUROC).

Results: From 1 January 2016 to 15 May 2019, there were 165 eligible patients, and 128 patients were included. In-hospital transfusion occurred in 97 (75.8%) of patients. Blood lactate was associated with ongoing RBC transfusion (aOR: 2.00; 95% confidence interval [CI]: 1.36–2.94). Blood lactate provided acceptable discriminative ability for ongoing transfusion (AUROC: 0.78; 95% CI: 0.70–0.86).

Conclusions: After excluding patients with early deaths, a quarter of those who had prehospital RBC transfusion had no further transfusion in hospital. Venous blood lactate appears to provide value in identifying such patients. Lactate levels after pre-hospital transfusion could be used as a biomarker for transfusion requirement after trauma.

Keywords

blood transfusion, emergency medicine, helicopter, prehospital, wounds and injuries

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Highlights

- After pre-hospital blood transfusion for major trauma, 97 patients (75.8%) received ongoing blood component therapy after hospital admission.
- Higher blood lactate, measured after pre-hospital blood transfusion, was associated with ongoing red blood cell transfusion.
- Lactate levels after pre-hospital transfusion could be used as a biomarker for activation of major haemorrhage protocols.

INTRODUCTION

Human blood is a precious resource. All blood for transfusions in Australia is voluntarily donated. It is estimated that one in three Australians require a blood transfusion in their lifetime. However, only 3% of Australians donate blood. This mismatch in supply and demand often leads to critical shortages in supply of blood components and is consistent from reports around the world [1].

The appropriate use of this precious resource is therefore paramount. The appropriate use of blood components is defined by transfusion only to treat a condition leading to significant morbidity or mortality that cannot be prevented or managed effectively by other means [2]. In the setting of critical bleeding, red blood cell (RBC) transfusion is often an essential, life-saving intervention. However, the indications to commence RBC transfusion remain unclear. Several scoring tools have been proposed, but none provide optimal diagnostic performance for routine clinical use [3–8]. Current practice, therefore, results in a substantial proportion of patients receiving potentially avoidable transfusions [9–12].

Decision-making for initiation of prehospital transfusion for critical bleeding has further unique challenges. Information about the pathophysiology of haemorrhage is often deficient, with incomplete characterization of the source of bleeding and volume of blood loss. Physiological responses are highly variable and do not always correlate with blood loss in some populations, such as in older patients and patients managed with sedative and paralytic medications [13]. Furthermore, physiological measurements such as blood pressure are usually performed through non-invasive means with potential for error [14].

Recently, pre-hospital blood lactate measurement has been proposed to predict the need for ongoing in-hospital blood transfusion [15]. However, this is currently not in routine practice. Prior to initiating additional investigations into pre-hospital care, we sought to explore the association of in-hospital lactate measurements and ongoing blood transfusion. Therefore, the aims of this study were to evaluate, among patients who had pre-hospital blood transfusion, variables, including lactate levels, with ongoing transfusion in hospital. We hypothesised that higher lactate levels would be associated with ongoing blood transfusions. This would inform a prospective trial of measuring lactate levels prior to and after pre-hospital transfusion.

MATERIALS AND METHODS

Setting

Ambulance Victoria provides emergency aeromedical service in the state of Victoria. It is the single provider of emergency aeromedical care in the state and operates five emergency helicopters from four bases across Victoria. The service conducts primary scene responses as well as inter-hospital transports and search and rescue operations, serving a population of almost 6.7 million people across an area of approximately 227,000 square kilometres. The helicopters are staffed by senior, post-graduate qualified intensive care flight paramedics, who each have at least a decade of pre-hospital experience. Each helicopter carries four units of group O RhD-negative RBC. Patients eligible for RBC are those with suspected haemorrhage and hypovolaemia after clinical judgement of the paramedic and, at the time of study, required approval from a clinician at the base. RBC is transfused to a target systolic blood pressure ≥70 mmHg, or if there is concurrent severe traumatic brain injury, a target systolic blood pressure ≥120 mmHg. There was no provision for whole blood transfusion during the time of this study.

Data sources

Eligible patients were identified from Ambulance Victoria clinical records, and data on demographics, initial (pre-hospital) vital signs, mechanisms of injury and pre-hospital management were extracted from the electronic records. Identifiers were used to match cases to hospital medical records, and an explicit medical records review was conducted to extract variables on hospital management that included vital signs, blood test results and initial transfusion of blood components.

Eligible patients

Patients were eligible for inclusion into this study if transported by Ambulance Victoria Helicopter Emergency Medical Service to The Alfred Hospital, one of two adult major trauma centres in Victoria between 1 January 2016 and 15 May 2019 and received at least one unit of pre-hospital RBC. Patients were excluded if they could not be

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matched to hospital records (incorrect name and date of birth), did not have a blood lactate recorded on arrival to the emergency department (ED) or died within 1 h of arrival to the ED and without any further transfusion.

Outcome variable

The primary outcome variable was transfusion of at least one unit of RBC within 4 h of arrival in hospital.

Exposure variables

The primary independent variable was a measure of venous blood lactate level measured immediately on hospital arrival. Blood lactate was not measured in the pre-hospital phase of care. First available vital signs were extracted. The heart rate and blood pressure were combined to calculate the shock index (SI) [16, 17].

Analysis

Continuous and near-normally distributed variables were summarized using mean (standard deviation; SD) and compared using Student's *t*-test. Continuous variables with skewed distribution and ordinal variables were summarized using median (interquartile range; IQR) and compared using Wilcoxon Rank Sum test. Count variables were summarized using percentages and compared using the chi-square test.

The association of pre-hospital variables and blood lactate with the primary outcome of hospital transfusion were explored using odds ratios and among independent variables that demonstrated an association with the primary outcome ($p < 0.1$), multi-variable logistic regression analysis was used to generate adjusted odds ratios (reported with 95% confidence intervals). The final model was assessed using Hosmer–Lemeshow goodness of fit to test whether or not the observed event rates matched expected event rates in the model population; small *p* values suggest that the model is a poor fit. The Variance Inflation Factor (VIF) was used as a measure of the severity of multi-collinearity in the regression analysis; small values (<3) indicate low correlation. The discriminative ability of blood lactate and SI to predict hospital transfusion were assessed using area under receiver operating characteristic curve (AUROC) and reported with 95% confidence intervals (CIs). AUROCs were compared using a test for the equality of the area under the curves and an algorithm proposed by De Long, et al. [18]. All analyses were conducted using Stata v 17.0 (College Station, TX, USA). *p* values <0.05 were defined to be statistically significant.

The study was approved by The Alfred Hospital Human Research and Ethics committee (study ID 4/20) and Ambulance Victoria Research Committee (Project ID 20–009). The requirement to seek informed consent from patients was waived.

RESULTS

From 1 January 2016 to 15 May 2019, there were 165 patients transported to The Alfred Hospital who received pre-hospital RBC. Of these, we excluded 37 patients; we were unable to link 21 patients' details to hospital medical records, 15 patients had no blood lactate measured, including eight who had early death and one additional patient died early on arrival, without the opportunity for further blood transfusion. This left 128 patients available for analysis (Figure 1).

Included patients were generally young with a mean age of 45.7 (SD 20.7) years, and the majority ($n = 103$; 80.5%) were male. The overall pre-hospital time was 156 (IQR 119–208) min, of which transport time was 34.5 (IQR 23–54) min. On initial measurement of vital signs, 71 (55.5%) patients had a SI >1.0. Patients received a median of 2 (IQR 1–4) units of pre-hospital RBC.

In the first 4 h of resuscitation, 97 (75.8%) patients were transfused further RBCs, and 31 (24.2%) did not receive further transfusion and formed the group who could potentially have pre-hospital transfusion avoided. Among the 31 patients who did not have further RBC transfusion, a total of 71 units of RBC had been transfused pre-hospital. A comparison of patients who had ongoing in-hospital transfusion versus those that did not is presented in Table 1.

The association of lactate levels and ongoing blood transfusion is illustrated in Figure 2. For every 1 mmol/L increase in lactate levels on arrival to the ED, the odds of hospital transfusion increased by 1.83; 95% CI: 1.35–2.47. Hospital transfusion was also associated with higher heart rate and SI, but not with a change in pre-hospital to in-hospital SI. (Table 2) When adjusted for age, mechanism of injury and pre-hospital SI, for every 1 mmol/L increase in blood lactate, the adjusted odds of in-hospital RBC transfusion increased by 2.0; 95% CI: 1.36–2.94. Detailed results of the regression model are available in

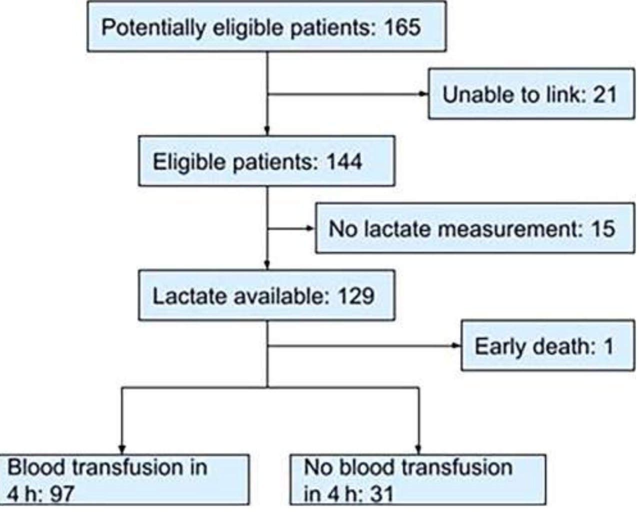

FIGURE 1 Selection of patients.

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TABLE 1 Pre-hospital patient characteristics sub-grouped by hospital transfusion.

	Ongoing hospital transfusion ($n = 97$)	No hospital transfusion in first 4 h ($n = 31$)	p value
Age			0.049
<25 years	20 (20.6%)	3 (9.7%)	
25–34 years	21 (21.7%)	3 (9.7%)	
35–49 years	24 (24.7%)	5 (16.1%)	
50–64 years	17 (17.5%)	10 (32.3%)	
≥65 years	15 (15.5%)	10 (32.3%)	
Sex			0.42
Male	76 (78.4%)	27 (87.1%)	
Female	21 (21.6%)	4 (12.9%)	
Total pre-hospital time, min; median (IQR)	147 (121–186)	170 (116–266)	0.08
Transport time, min; median (IQR)	32 (23–49)	45 (25–75)	0.07
Mechanism of injury			0.011
Motor vehicle crash	73 (75.3%)	17 (54.8%)	
High fall	2 (2.1%)	5 (16.1%)	
Low fall	6 (6.2%)	4 (12.9%)	
Pedestrian	0	1 (3.2%)	
Penetrating	7 (7.2%)	2 (6.4%)	
Other	9 (9.3%)	2 (6.4%)	
Initial SBP, mmHg; mean (SD)	98 (28)	110 (27)	0.039
Initial SBP category			0.048
≤70 mmHg	20 (20.6%)	5 (16.1%)	
71–100 mmHg	36 (37.1%)	6 (19.3%)	
101–120 mmHg	21 (21.6%)	6 (19.3%)	
>120 mmHg	20 (20.6%)	14 (45.2%)	
Initial heart rate, beats/min; mean (SD)	106 (29)	103 (27)	0.62
Initial shock index, $b{\cdot}min^{-1}{\cdot}mmHg^{-1}$; mean (SD)	1.2 (0.55)	0.95 (0.40)	0.034
Initial shock index >1	57 (58.8%)	14 (45.2%)	0.18
Initial Glasgow Coma Scale[a]			0.32
3–8	58 (63.0%)	16 (51.6%)	
9–12	3 (3.3%)	0	
13–15	31 (33.7%)	15 (48.4%)	
Pre-hospital intubation[b]	57 (62.0%)	16 (51.6%)	0.31
Volume of pre-hospital RBC, units; medians (IQR)	2 (1–4)	2 (1–3)	0.60

Abbreviations: IQR, interquartile range; RBC, red blood cell; SBP, systolic blood pressure; SD, standard deviatiion.
[a]Missing data or five patients.
[b]Missing data for eight patients.

Supplementary, Appendix 1, with Hosmer–Lemeshow goodness-of-fit p value of 0.87 and mean VIF 1.40.

The discriminative abilities of blood lactate (as a continuous variable), blood lactate (categorized to whole number intervals) and the SI are illustrated in Figure 3. The AUROC for blood lactate (continuous) was 0.78 (95% CI: 0.70–0.86), which was similar to blood lactate (categorized) with an AUROC of 0.76 (95% CI: 0.66–0.85); $p = 0.06$. The AUROC for pre-hospital SI to discriminate patients administered hospital RBC was 0.63 (95% CI: 0.52–0.75), which was significantly poorer compared with the AUROC for blood lactate (continuous; $p = 0.008$).

DISCUSSION

After exclusion of patients who had early death after arrival to hospital, a quarter of patients who received pre-hospital blood did not have ongoing RBC transfusion in the first 4 hours in hospital. In-hospital

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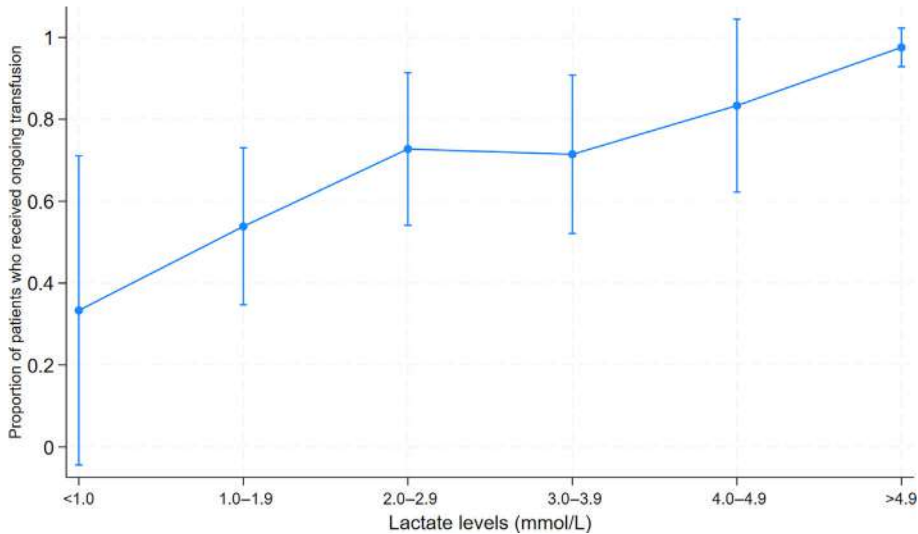

FIGURE 2 Lactate levels and proportion of patients who had ongoing blood transfusion.

TABLE 2 Venous blood lactate and vital signs on arrival to hospital.

	Ongoing hospital transfusion ($n = 97$)	No hospital transfusion in first 4 h ($n = 31$)	*p* value
Blood lactate, continuous; mmol/L; mean (SD)	4.9 (3.9)	2.2 (1.2)	<0.001
Blood lactate, categorized; mmol/L			<0.001
≤1	4 (4.1%)	5 (16.1%)	
>1.0–2.0	13 (13.4%)	11 (35.5%)	
>2.0–3.0	18 (18.6%)	7 (22.6%)	
>3.0–4.0	13 (13.4%)	5 (16.1%)	
>4.0	49 (50.5%)	3 (9.7%)	
First hospital SBP, mmHg; mean (SD)	113 (41)	127 (21)	0.06
First hospital HR, b/min; mean (SD)	111 (30)	97 (25)	0.019
First hospital SI, b·min^{-1}·mmHg^{-1}; mean (SD)	1.10 (0.42)	0.78 (0.26)	<0.001
Increase in SI	38 (39.2%)	9 (29.0%)	0.18

Abbreviations: HR, heart rate; SBP, sytolic blood pressure; SD, standard deviation; SI, shock index.

blood lactate was associated with ongoing transfusion with acceptable discriminative ability to differentiate between those that had hospital RBC transfusion and those that did not. This generates the hypothesis that an early measure of blood lactate levels could be used to determine the need for blood transfusion.

Avoidable blood transfusion continues to occur in a substantial proportion of injured patients [12]. A key determinant of avoidable transfusions is the absence of reliable clinical criteria for predicting transfusions [19]. Clinical gestalt appears to outperform most clinical scores, and this study generates the hypothesis that blood lactate levels could be considered in decision making for the initiation of major haemorrhage protocols [6].

Lactatemia is likely a reflection of anaerobic respiration that occurs during haemorrhagic shock and has been previously shown to have predictive value for mortality after trauma [20]. In isolation, lactate levels are therefore indicators of the severity of tissue hypoxia during haemorrhagic shock. Effective circulatory resuscitation is therefore expected to clear blood lactate levels, with both initial lactate levels and early lactate clearance being associated with outcomes [21, 22].

It should be stressed that blood lactate should not be the only biomarker or indicator for pre-hospital transfusion. Current physiological markers of systolic blood pressure, combined with clinical assessment of ongoing haemorrhage are likely to have ongoing importance in transfusion decision-making. Permissive hypotension to a systolic blood pressure of 70 mmHg in the absence of head injury appears to be at the limit of tolerance. However, it is possible that the targets for systolic blood pressure of 120 mmHg in the setting of critical bleeding and suspected traumatic brain injury could be lowered. However, current evidence does not provide confident targets and further trials on this topic are indicated [23].

This study is further limited by potential selection bias of patients who arrived alive in hospital. It is possible that ongoing improvements in pre-hospital care using blood components, particularly for those

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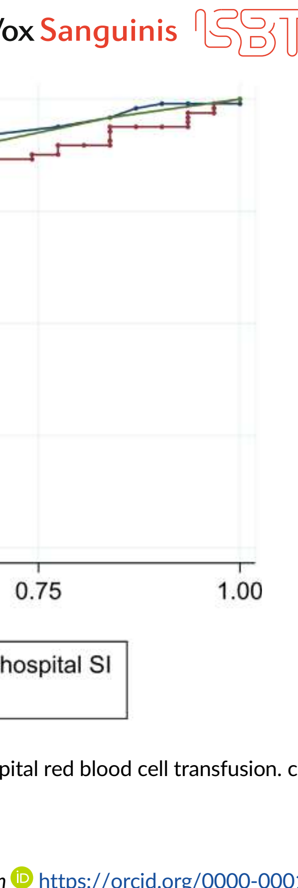

FIGURE 3 Discriminative ability of blood lactate and pre-hospital shock index (SI) for hospital red blood cell transfusion. cat, lactate treated as a categorical variable; cont, lactate treated as a continuous variable.

with long pre-hospital times, could improve survival [24]. In future studies, the addition of such critically bleeding patients who are expected to have high blood lactate could further improve specificity of lactate for transfusion. Data on clinical gestalt of paramedics were not collected and could provide better diagnostic capacity for ongoing blood product transfusion.

In conclusion, among critically bleeding patients transfused with prehospital RBC, a quarter did not have further transfusion in hospital. The measurement of pre-hospital and pre-transfusion venous blood lactate appear promising as a biomarker to augment decisions to initiate or continue blood transfusion.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data for this manuscript are available upon reasonable request and subject to ethic committee approval.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Evaluation of the impact of diagnostic blood loss and red blood cell transfusion in very-low-birth-weight anaemic neonates during hospitalization: A multi-centre retrospective clinical study

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Abstract

Background and Objectives: Diagnostic blood loss is a significant factor in the development of anaemia in neonates with very low birth weight. This study aimed to assess the clinical efficacy of intervention approaches involving varying diagnostic blood loss and red blood cell transfusion volumes in neonates with very low birth weights experiencing anaemia during hospitalization.

Materials and Methods: A total of 785 newborns with anaemia weighing less than 1500 g were enrolled from 32 hospitals in China. The study involved monitoring diagnostic blood loss and red blood cell transfusion and evaluating relevant interventions such as red blood cell transfusion and clinical outcomes. Three intervention approaches were established based on the difference between blood loss and transfusion (Intervention Approaches 0, 1 and 2). The primary outcomes measured were unsatisfactory weight gain during hospitalization and neonatal mortality. The secondary outcomes included related complications.

Results: In the non-hospital-acquired anaemia group, Intervention Approach 2 had the highest incidence of below-normal weight gain (odds ratio [OR]: 3.019, 95% confidence interval [CI]: 1.081–8.431, $p = 0.035$). Multivariate analysis revealed that Intervention Approach 1 had a protective effect on weight gain. In the hospital-acquired anaemia group, Intervention Approach 2 had the highest incidence of below-normal weight gain (OR: 3.335, 95% CI: 1.785–6.234, $p = 0.000$) and

Ting Ma and Jiangcun Yang contributed equally to this paper and should be considered as co-first authors.

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mortality (OR: 5.341, 95% CI: 2.449–11.645, $p = 0.000$), while Intervention Approach 1 had the lowest incidence of intraventricular haemorrhage. Intervention Approach 1 demonstrated favourable outcomes in both anaemia groups.

Conclusion: Intervention Approach 1 improved weight gain and reduced mortality and complications in both the non-hospital-acquired and hospital-acquired anaemia groups.

Keywords

diagnostic blood loss, hospital-acquired anaemia, neonates, transfusion

Highlights

- This retrospective, multi-centre study focuses on very-low-birth-weight neonates in China. During hospitalization, a comprehensive assessment of patient characteristics and neonatal outcomes was conducted.
- The study aimed to determine the best blood transfusion intervention approach for diagnosing blood loss in very-low-birth-weight neonates. Three intervention approaches were categorized based on the difference between blood loss and red blood cell (RBC) transfusion volumes.
- Intervention Approach 1 showed promising results. The study provides insights into optimizing RBC transfusion for anaemic neonates, which may lead to improved prognosis and reduced complications.

INTRODUCTION

Very-low-birth-weight (VLBW) infants often develop complex underlying conditions during their hospital stay. Additionally, the neonatal blood system undergoes physiological changes, leading to a decrease in haemoglobin levels after birth. VLBW infants also have a low blood volume. Laboratory tests are essential for the accurate diagnosis and treatment of newborns. Timely correction of iatrogenic blood loss-induced anaemia is crucial for effective treatment and prognosis.

Transfusion rates are generally high among VLBW infants. Studies have reported transfusion rates of 94%, 62% and 35% in infants born at 24–28 weeks, 28–30 weeks and 30–32 weeks, respectively [1]. Some studies have reported transfusion rates as high as 80% in VLBW infants [2]. Diagnostic blood loss is inevitable during neonatal hospitalization. Although strategies for reducing blood loss through improved blood collection techniques have been proposed, their implementation in clinical practice remains challenging. Diagnostic blood loss in VLBW infants can lead to neonatal anaemia due to their low blood volume.

Hospital-acquired anaemia (HAA) refers to the development of anaemia during hospitalization in patients who are not anaemic upon admission [3, 4]. The occurrence of HAA is associated with prognosis and mortality [5]. Therefore, timely correction of anaemia in VLBW neonates is crucial. Inadequate blood transfusion can negatively affect neonatal development and treatment of primary diseases, and may lead to apnoea, neurological disorders or poor weight gain. However, excessive blood transfusion can result in complications such as iron overload, excessive circulatory stress and adverse reactions [6–9].

Previous studies have mainly focused on the relationship between diagnostic iatrogenic blood loss and neonatal anaemia. Most studies have aimed to reduce the frequency of blood collection or to improve collection methods; however, these are difficult to implement in clinical practice. Frequent blood tests for diagnosis or prevention of anaemia can lead to iatrogenic blood loss. Therefore, our objective was to identify the optimal intervention approach for diagnosing iatrogenic blood loss and guiding blood transfusion during hospitalization of VLBW newborns with anaemia.

STUDY DESIGN AND METHODS

Design and patient population

A multi-centre retrospective study was conducted across 32 hospitals in China, involving hospitalized newborns weighing less than 1500 g between January 2017 and June 2018. This study used a database of 785 VLBW newborns from 32 hospitals across 18 provinces and cities (Figure 1). This study was conducted in accordance with the principles of the Declaration of Helsinki. The study process is illustrated in Figure 2.

Data acquisition

Data organization and analysis were performed at Shaanxi Provincial People's Hospital using a tabular method. The database included

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FIGURE 1 Flow chart demonstrates the steps followed in the study. HAA, hospital-acquired anaemia; Hb, haemoglobin; HNAA, non-hospital-acquired anaemia; RBC, red blood cell; VLBW, very-low-birth-weight.

information on 785 VLBW newborns who received blood transfusions at the hospital.

Maternal information (age, delivery method, multiple births, pregnancy history, parity and maternal complications) and demographic characteristics of the newborns (sex, birth date, admission date, discharge date, birth weight, birth length, birth head circumference, admission weight, Apgar score at 1, 5 and 10 min, and accompanying admission diseases) were collected. Clinical data including in-hospital haemoglobin levels, laboratory-diagnosed blood loss during hospitalization, blood transfusion treatment (timing, volume, and type of blood products), body weight at discharge, mortality and complications were also included. The main outcome measures were the number of patients with below-normal weight gain and mortality, and the secondary outcome measures were retinopathy of prematurity, necrotizing enterocolitis and intraventricular haemorrhage. In our study, we compared the actual rate of weight gain in neonates with the normal rate specified in the Practical Neonatology Guidelines for different gestational ages. Patients with weight gain below the normal value were identified as having unsatisfactory growth, indicating below-normal weight gain. Conversely, patients with weight gain above the normal value were classified as having satisfactory weight gain.

Blood loss at each diagnostic laboratory was estimated based on a routine clinical test protocol. The assigned volumes were as follows: 1 mL for blood routine tubes, 1 mL for serum chemical laboratory tubes, 2 mL for coagulation tubes, 0.5 mL for arterial blood gas tubes, 1 mL for blood cultures and 1 mL for other diagnostic blood tests.

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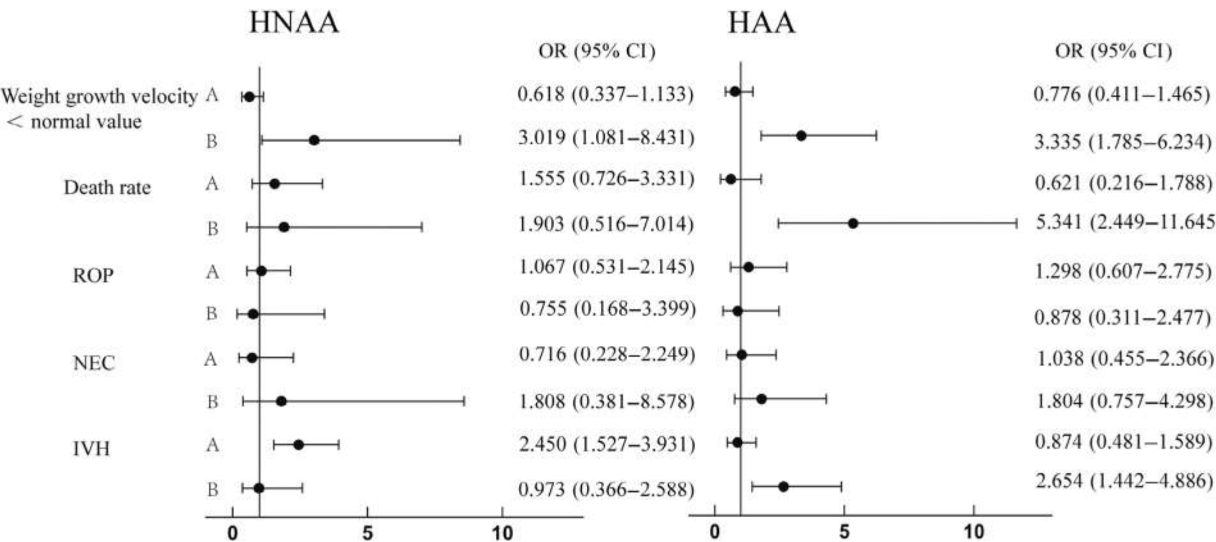

FIGURE 2 Risk analysis of outcome indicators using univariate analyses. Whisker bars reflect 95% confidence interval. Classification stage A: Intervention Approach 0 versus Intervention Approach 1, B: Intervention Approach 0 versus Intervention Approach 2. CI, confidence interval; HAA, hospital-acquired anaemia; HNAA, non-hospital-acquired anaemia; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; OR, odds ratio; ROP, retinopathy of prematurity.

Ethics approval and consent to participate

We obtained a waiver for informed consent from the ethics committee. Ethical approval for the study was obtained from the Medical Ethics Committee of Shaanxi Provincial People's Hospital (No: 2020-R001).

Definition and grouping

Definition

Haemoglobin (Hb) values of newborns on admission and during hospitalization were compared with the values specified in Practical Neonatology (5th edition) for VLBW infants with anaemia.

Non-hospital-acquired anaemia group (HNAA): Newborns with Hb values lower than normal upon admission, indicating anaemia at admission [3].

Hospital-acquired anaemia group (HAA): Newborns with Hb values within the normal range upon admission, but later dropping below normal during their hospital stay, indicating the development of anaemia during hospitalization.

Blood transfusion intervention approaches

Based on the description of neonatal blood loss and transfusion treatment in Chinese Blood Transfusion and Practical Neonatology (5th edition), three intervention approaches were categorized according to the difference between blood loss and transfusion:

Intervention Approach 0: The volume of transfused red blood cells was greater than the volume of iatrogenic blood loss from the phlebotomy.

Intervention Approach 1: The volume of transfused red blood cells was less than the volume of iatrogenic blood loss from the phlebotomy, with a difference of <10% of the newborn's total blood volume.

Intervention Approach 2: The volume of transfused red blood cell was less than the volume of iatrogenic blood loss from the phlebotomy, with a difference of more than 10% from the newborn's total blood volume.

Statistics

IBM SPSS version 24 was used for statistical analysis. Descriptive statistics presented median with interquartile range for continuous data based on data distribution characteristics. Categorical variables were summarized as numbers and percentages. Independent *t*-tests, Mann–Whitney tests or χ^2 tests were employed to compare group characteristics. A *p* value of less than 0.05 was considered statistically significant.

Correlations were analysed using univariate and multivariate logistic regression. Significant risk factors for below-normal weight growth rate and adverse outcomes (mortality, ROP, NEC, IVH) were identified, including intervention approach, gestational age, Hb levels, admission weight, Apgar score and co-morbidities. *p* Value <0.05 was considered significant. Odds ratio (OR) and 95% confidence interval (CI) were calculated.

RESULTS

Data distribution

A total of 766 neonates from 32 hospitals in China were included in the study after excluding cases with incomplete data. Among the hospitals, 12 dedicated to women's and children's health, while 20 were

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general hospitals. The distribution of cases in each province is shown in Figure 1.

Patient characteristics

Tables 1 and 2 present the characteristics of the newborns and mothers on admission in the different groups. There were no statistically significant differences in the baseline distribution of delivery mode, incidence of multiple births, number of pregnancies, parity counts or associated complications (Table 1).

In the HNAA group, there were 248 newborns in Intervention Approach 0, 105 in Intervention Approach 1 and 22 in Intervention Approach 2. The proportions of males were 50.8%, 55.2% and 63.6% in the three approaches, respectively. The median gestational age of the infants was 29 weeks. The median birth lengths were 39 cm (interquartile range [IQR], 36–40), 38 cm (IQR, 35–40) and 37.5 cm (IQR, 35–41) in the different approaches, respectively. The median birth weights were 1270 g (IQR, 1100–1400), 1140 g (IQR, 1000–1320) and 1200 g (IQR, 958–1343), respectively.

In the HAA group, there were 202 newborns in Intervention Approach 0, 122 in Intervention Approach 1 and 67 in Intervention Approach 2. The proportions of males were 53.5%, 54.1%, and 62.7% in the three approaches, respectively. The median gestational age was 30 weeks, and the median birth lengths were 39 cm (IQR, 37–40), 39 cm (IQR, 36–41) and 40 cm (IQR, 36–42), respectively. The median birth weights were 1231 g (IQR, 1100–1360), 1216.5 g (IQR, 1115–1350) and 1260 g (IQR, 1130–1390), respectively. The specific characteristics of the neonates upon admission are shown in Table 2.

Characteristics of diagnostic blood loss and erythrocyte transfusion

Table 3 presents the characteristics of diagnostic iatrogenic blood loss and red blood cell (RBC) transfusion. In the HNAA group, the median blood loss volumes were 9 mL/kg (IQR, 8–11), 9 mL/kg (IQR, 8–10) and 17 mL/kg (IQR, 10–21) in the different approaches. The number of patients treated with RBC transfusion was 248 (100%), 89 (84.8%) and 12 (54.5%), respectively.

In the HAA group, the median blood loss volume was 9 mL/kg (IQR, 8–10), 9 mL/kg (IQR, 8–10), and 10 mL/kg (IQR, 9–16) in the different approaches. RBCs were transfused in 202 (100%), 98 (80.3%) and 25 (37.3%) patients in the different approaches. Notably, Intervention Approach 2 had the highest total blood loss but the lowest proportion of erythrocyte transfusions.

Outcome

Positive cases were defined as those with lower-than-normal weight gain. The number and proportion of positive cases were among the primary outcome measures.

TABLE 1 Basic demographic characteristics of mothers.

		HNAA				HAA			
		Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value	Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value
Model of delivery, *n* (%)	Vaginal delivery	112 (45.3)	48 (45.7)	11 (50.0)	0.916	56 (27.7)	34 (27.9)	25 (37.3)	0.296
	Caesarean section	135 (54.7)	57 (54.3)	11 (50.0)		146 (72.3)	88 (72.1)	42 (62.7)	
Number of births, *n* (%)	Single foetus	165 (67.3)	65 (62.5)	14 (63.6)	0.667	139 (69.5)	87 (71.9)	48 (71.6)	0.882
	Multiple births	80 (32.7)	39 (37.5)	8 (36.4)		61 (30.5)	34 (28.1)	19 (28.4)	
Number of pregnancy, *n* (%)	1	86 (35.5)	38 (37.3)	6 (27.3)	0.675	63 (31.5)	41 (33.9)	21 (31.3)	0.894
	≥2	156 (64.5)	64 (62.7)	16 (72.7)		137 (68.5)	80 (66.1)	46 (68.7)	
Parity, *n* (%)	1	119 (48.4)	58 (55.8)	10 (47.6)	0.434	96 (48.0)	51 (42.1)	28 (41.8)	0.496
	≥2	127 (51.6)	46 (44.2)	11 (52.4)		104 (52.0)	70 (57.9)	39 (58.2)	
Number of mothers with complications, *n* (%)	1	202 (81.5)	83 (79.0)	19 (86.4)	0.703	166 (82.2)	100 (82.0)	45 (67.2)	0.022
	≥2	46 (18.5)	22 (21.0)	3 (13.6)		36 (17.8)	22 (18.0)	22 (32.8)	

Abbreviations: HAA, hospital-acquired anaemia; HNAA, non-hospital-acquired anaemia.

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TABLE 2 Clinical characteristics of all neonates in this study, $n = 766$.

		HNAA				HAA			
		Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value	Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value
Total (*n*)		248	105	22		202	122	67	
Sex, *n* (%)	Male	126 (50.8)	58 (55.2)	14 (63.6)	0.431	108 (53.5)	66 (54.1)	42 (62.7)	0.402
	Female	122 (49.2)	47 (44.8)	8 (36.4)		94 (46.5)	56 (45.9)	25 (37.3)	
Gestational age (week), median (IQR)		29a (28–31)	29b (28–30)	29c (27–29)	0.009	30a (28–31)	30b (29–32)	30 (29–31)	0.025
Birth length (cm), median (IQR)		39a (36–40)	38b (35–40)	37.5 (35–41)	0.091	39 (37–40)	39 (36–41)	40 (36–42)	0.561
Birth head circumference (cm), median (IQR)		28a (26–29)	26b (25–28)	26 (25–28)	0.001	28 (26–29)	28 (26–29)	28 (27–30)	0.339
Admission weight (g), median (IQR)		1270a (1100–1400)	1140b (1000–1320)	1200 (958–1343)	0.001	1231 (1100–1360)	1216.5 (1115–1350)	1260 (1130–1390)	0.561
Apgar score, median (IQR)	1 min	8a (6–9)	8b (7–10)	5c (4–7)	0.000	8 (6–9)	8a (6–9)	7b (5–9)	0.106
	5 min	9a (8–10)	10b (8–10)	8c (7–8)	0.000	9 (8–9)	9a (8–10)	9b (7–9)	0.103
	10 min	10a (9–10)	10 (9–10)	9b (7–9)	0.005	9 (8–10)	10 (8–10)	9 (8–10)	0.128
No. of concomitant disease median (IQR)		4 (3–6)	4 (4–5)	4 (3–5)	0.763	5 (4–6)	4 (3–5)	5 (3–7)	0.061

Abbreviations: HAA, hospital-acquired anaemia; HNAA, non-hospital-acquired anaemia; IQR, interquartile range.

TABLE 3 Characteristics of diagnostic blood loss and blood transfusion.

		HNAA				HAA			
		Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value	Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value
Bleeding volume (mL/kg), median (IQR)		9 (8–11)	9 (8–10)	17 (10–21)	0.000	9 (8–10)	9 (8–10)	10 (9–16)	0.000
Mean time between venotomy (day), median (IQR)		2 (1–4)	2 (1–4)	1 (1–3)	0.254	3 (1–4)	4 (2–5)	2 (1–4)	0.009
Hb level (g/L), median (IQR)	Admissions	136 (99–151)	110 (88–144)	143 (129–155)	0.000	176 (166–189)	181 (170–194)	188 (176–199)	0.000
	Before blood transfusion	103 (89–125)	98 (85–113)	140 (99–152)	0.001	118 (96–165)	122 (96–168)	168 (114–188)	0.000
	The lowest value during hospitalization	90 (84–98)	86 (81–95)	101 (81–118)	0.028	94 (85–103)	95 (84–105)	99 (86–122)	0.153
No. of erythrocyte transfusion cases, *n* (%)		248 (100)	89 (84.8)	12 (54.5)	0.000	202 (100)	98 (80.3)	25 (37.3)	0.000
Frequency erythrocyte transfusion, *n* (%)		2 (2.3)	2 (1.4)	1 (0.2)	0.000	2 (1.3)	1 (1.2)	0 (0.1)	0.000
Total amount of erythrocyte transfusion		60 (41.90)	42 (22.71)	11 (0.45)	0.000	50 (35.72)	30 (20.50)	0 (0.26)	0.000
No. of Hb < normal value on discharge, *n* (%)		107 (43.1)	34 (32.4)	14 (63.6)	0.016	103 (51)	66 (54.1)	40 (59.7)	0.457

Abbreviations: HAA, hospital-acquired anaemia; HNAA, non-hospital-acquired anaemia; IQR, interquartile range.

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TABLE 4 Analysis of outcome indicators of neonatal blood transfusion.

	HNAA				HAA			
	Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value	Intervention Approach 0	Intervention Approach 1	Intervention Approach 2	*p* Value
Weight growth rate < normal value, *n* (%)	54 (24.9)	17 (17.0)	8 (50.0)	0.012	35 (19.2)	17 (15.6)	27 (44.3)	0.000
No. of death, *n* (%)	19 (7.7)	12 (11.4)	3 (13.6)	0.394	13 (6.4)	5 (4.1)	18 (26.9)	0.000
ROP, *n* (%)	29 (11.7)	13 (12.4)	2 (9.1)	0.908	17 (8.4)	13 (10.7)	5 (7.5)	0.709
NEC, *n* (%)	13 (5.2)	4 (3.8)	2 (9.1)	0.576	16 (7.9)	10 (8.2)	9 (13.4)	0.368
IVH, *n* (%)	69 (27.8)	51 (48.6)	6 (27.3)	0.001	37 (18.3)	20 (16.4)	25 (37.3)	0.001

Abbreviations: HAA, hospital-acquired anaemia; HNAA, non-hospital-acquired anaemia; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity.

In the HNAA group, 54 (24.9%), 17 (17%) and 8 (50%) positive cases were observed in the different approaches, respectively. The number of positive cases in Intervention Approach 2 was significantly higher than those in the other two approaches. There was no significant difference in mortality rates. Secondary outcomes included major complications, and the incidence of intraventricular haemorrhage (IVH) was lower in Intervention Approach 0 (27.8%) (Table 4).

In the HAA group, 35 (19.2%), 17 (15.6%) and 27 (44.3%) positive cases were detected in the different approaches, respectively. The number of positive cases in Intervention Approach 2 was significantly higher than those in the other two approaches. The mortality rate in Intervention Approach 2 was 26.9%, which was significantly higher than those in the other two approaches. The incidence of IVH was lower in Intervention Approach 1 (16.4%) (Table 4).

Univariate logistic regression analysis in the HNAA group showed that Intervention Approach 2 was associated with a higher risk of a positive event (OR: 3.019, 95% CI: 1.081–8.431, $p = 0.035$). Intervention Approach 1 was a risk factor for the occurrence of IVH compared with Intervention Approach 0 (OR: 2.45, 95% CI: 1.527–3.931, $p = 0.035$). No significant differences were observed in other variables (Figure 2). In the HAA group, Intervention Approach 2 was associated with a higher risk of a positive event (OR: 3.335, 95% CI: 1.785–6.234, $p = 0.000$). Intervention Approach 2 was also associated with a higher risk of mortality (OR: 5.341, 95% CI: 2.449–11.645, $p = 0.000$). Intervention Approach 2 was a risk factor for the incidence of IVH compared with Intervention Approach 0 (OR: 2.654, 95% CI: 1.442–4.886, $p = 0.002$) (Figure 2).

Multivariate logistic regression analysis showed that in the HNAA group, large gestational age, Intervention Approach 1 and Apgar score 5 min (≥7) were protective factors for a positive event. In the HAA group, Intervention Approach 0 and large gestational age were protective factors for a positive event, and there was no difference between Intervention Approach 0 and 1 (Table S1).

DISCUSSION

This study aimed to evaluate the correlation between different intervention approaches of RBC transfusion and the occurrence of positive events (below-normal weight gain and mortality) and major complications (ROP, NEC and IVH) in newborns with varying anaemia statuses following hospital admission due to iatrogenic blood loss. The newborns were grouped accordingly. After correcting for baseline confounders, Intervention Approach 1 was identified as the optimal intervention in the HNAA group, in which the total volume of blood loss was limited to within 10% of the total blood volume after transfusion, resulting in the best prognosis. In the HAA group, the combined results of the primary and secondary outcome measures demonstrated improved weight gain, reduced mortality and fewer complications with the intervention of Intervention Approach 1.

Diagnostic blood loss in very-low-birth-weight newborns with VLBW contributes significantly to anaemia during hospitalization owing to their physiological vulnerability. Prevention and treatment should focus on minimizing unnecessary examinations and optimizing blood sampling [10–12]. In vitro devices can reduce blood loss but may increase costs and affected test quality. An alternative strategy involves big data analysis and screening to diagnose high-risk patients, thereby reducing unnecessary tests. However, this approach was mainly used in adult ICU patients, and eliminating false negatives remains challenging [13–15].

Our study aimed to present an intuitive approach for exploring an optimal intervention approach for clinical blood transfusion. By conducting laboratory tests based on the physician's advice and considering the accumulated blood loss and neonatal blood volume, we treated neonatal anaemia independently of the laboratory test results. We also discussed appropriate blood transfusion schemes to avoid the risk of complications associated with excessive blood transfusion.

Previous studies on the cumulative volume of blood loss in preterm infants reported a range of 14–90 mL/kg [16, 17]. Our study showed a cumulative blood loss volume of between 9 and 17 mL/kg, which is consistent with the range reported in correlational studies in China. The characteristics of blood loss in the first few days after hospitalization were consistent with previous findings [18]. The discrepancies in the reported blood loss can be attributed to the population and region, choice of experimental project type, clinical nursing practices and the blood volume required by the specific test equipment.

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Diagnostic blood loss directly correlates with a decrease in haemoglobin or haematocrit levels [19–21]. Increased blood loss in VLBW infants directly leads to a higher blood transfusion rate. In our study, Intervention Approach 2 showed greater blood loss than the other two approaches; however, the proportion of infused red blood cells was the lowest. Evaluating the proportion of newborns with persistently lower-than-normal haemoglobin levels at discharge, we found that the incidence of abnormal haemoglobin cases was 63.6% and 59.7% in the HNAA and HAA groups, respectively, when applying Intervention Approach 2. There was a significant difference in incidence between Intervention Approach 2 and the other approaches in the HNAA group. Inadequate blood transfusion regimens can result in poor clinical outcomes and an increased risk of anaemia in newborns.

HAA was associated with increased in-hospital mortality, particularly severe HAA, which significantly affected the prognosis after discharge [22–26]. Our research findings demonstrated that the implementation of Intervention Approach 2 resulted in a higher prevalence of inadequate weight gain among VLBW infants in both the HNAA and HAA groups. This trend negatively affected the overall growth and development of newborns. Intervention Approach 2 also showed the highest mortality rate in the HAA group. These findings highlight the importance of a reasonable RBC transfusion intervention approach that benefits the growth and development of VLBW infants while avoiding the adverse effects of excessive or inadequate blood transfusion schedules.

Blood transfusion therapy is necessary to treat anaemia in VLBW neonates; however, excessive transfusion can lead to complications that affect treatment outcomes and long-term growth and development [22]. Red blood cell transfusion disrupts microcirculation regulation and affects tissue oxygenation [27]. Blood transfusion has been linked to the incidence of NEC, IVH and ROP.

A study conducted on premature infants in Iowa revealed that restricted blood transfusion was associated with periventricular leukomalacia and IVH, whereas free red blood cell transfusion was potentially neuroprotective [28]. Research on transfusion-induced IVH had yielded mixed results, with some studies showing an increased incidence of IVH with blood transfusion, whereas a few reports have suggested that preventing anaemia is beneficial for IVH [29, 30]. Our results indicated no significant differences in NEC and ROP between the HNAA and HAA groups under different intervention approaches. In Intervention Approach 1, the incidence of IVH increased in the HNAA group, but was the lowest in the HAA group. These findings suggested that despite selecting the Intervention Approach 1 intervention based on the main outcome, IVH should still be considered in the HNAA group, considering the different neonatal baseline conditions. Therefore, it was necessary to determine the most appropriate transfusion frequency and volume to reduce the occurrence of IVH in this intervention approach. In the HAA group, the Intervention Approach 1 intervention demonstrated better main outcomes and fewer complications.

In conclusion, this study aimed to investigate the optimal blood transfusion intervention approach for diagnosing blood loss in very-low-birth-weight neonates with VLBW. After correcting for baseline confounders, Intervention Approach 1 was identified as the optimal intervention in the HNAA group, in which a total blood loss of less than 10% of the total blood volume after transfusion led to the best prognosis. In the HAA group, the Intervention Approach 1 intervention showed a better prognosis with improved growth rate, lower mortality and fewer complications, as indicated by a combination of primary and secondary outcome indicators.

This study has some limitations. This study included only VLBW newborns who received blood transfusions and excluded those who did not. However, because 80% of newborns with VLBW received blood transfusions, we believed that the data from this study fairly represent the overall situation of newborns with VLBW. In addition, Intervention Approach 1 demonstrated favourable primary outcomes in the HNAA group but showed a higher incidence of IVH. Therefore, in future studies, we aim to investigate the frequency, interval and volume of blood transfusions to improve the secondary outcomes in this intervention approach.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis

Trends in category and grade for therapeutic plasma exchange in the latest guideline on therapeutic apheresis by the American Society for Apheresis: Hurdles in pursuing evidence-based medicine

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Abstract

Background and Objectives: The Writing Committee of American Society for Apheresis released the ninth edition of guidelines for therapeutic apheresis in 2023. Categories have been a part of the guidelines since the first edition, and the grading system was introduced in the fifth edition, with updates in every new edition. In this study, we investigated the category and grade change trends through the latest five editions, focusing on therapeutic plasma exchange, to suggest future directions as part of evidence-based medicine.

Materials and Methods: Categories and grades for therapeutic plasma exchange (TPE) were collected and analysed from the fifth through ninth editions. We aligned classification changes to the ninth edition's clinical context and compared its categories and grades with those introduced in the guideline.

Results: Among 166 total indications in the ninth edition, 118 included TPE procedure, either as a sole treatment or as one of the therapeutic apheresis techniques. The total number of indications changed, but Category III remained predominant throughout the editions. Similarly, Grade 2C consistently emerged as the most prevalent grade. Notably, 24 cases had grade changes. Of the 16 cases with evidence quality changes, the quality weakened in six and improved in 10. Evidence levels were not improved throughout the study period for 102 clinical conditions.

Conclusion: To address gaps in evidence quality, international collaboration is imperative to establish comprehensive large-scale studies or randomized controlled trials. This will refine the use of therapeutic apheresis, including TPE, to foster evidence-based advancements in clinical practice.

Keywords

ASFA guidelines, evidence-based medicine, therapeutic apheresis, therapeutic plasma exchange

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Highlights

- In the fifth through to the ninth editions of the American Society for Apheresis (ASFA) guidelines, Category III and Grade 2C were the most predominant.
- The utilization of therapeutic plasma exchange (TPE) is increasing, but due to its infrequent application in rare situations, accumulating substantial evidence is challenging. To implement TPE, most decisions rely on individual patients' clinical conditions.
- International medical professionals should be aware of the limitations of the ASFA guidelines when applying them. In pursuit of evidence-based medicine, international collaboration is needed to conduct large-scale studies and randomized controlled trials.

INTRODUCTION

Therapeutic apheresis (TA) refers to extracorporeal therapy in which blood is manipulated outside the body and then returned to the patient. Since its initial description by Abel et al. almost 100 years ago in 1914, apheresis has progressed in tandem with other medical technologies [1]. At present, TA is being used with diverse modalities for numerous indications, but there are limited guidelines available. The guidelines by the Writing Committee of the American Society for Apheresis (ASFA) are globally recognized and widely adopted [2]. The ASFA guidelines, which were first issued in 1986 [3], have been revised every 3 years; the ninth edition is the most recent.

The Writing Committee has updated various TA indications using an evidence-based approach through evidence grading and indication categorization. Categories have been part of the guidelines since the first edition. The Grade system, introduced in the fifth edition in 2010, was adopted from the American College of Chest Physicians Evidence-Based Clinical Practice Guidelines and replaced the previous 'strength of evidence' component with a quality of evidence evaluation [4–6].

Upon the release of new guidelines, the categories and grades of existing indications have changed and indications have been added and removed. The aforementioned decisions were made based on the quality of evidence in the published literature. The ASFA guidelines advocate an evidence-based approach; thus, with each new edition, the number of indications with higher levels of evidence has increased.

In this study, our primary focus was to track the changes in the categories and grades of the ASFA guidelines, particularly concerning the therapeutic plasma exchange (TPE) procedure. Furthermore, based on the trends, we aimed to provide insights into the future direction of the ASFA guidelines.

MATERIALS AND METHODS

We collected and reviewed the categories and grades of indications corresponding to the TPE method from the ASFA guidelines, covering from the 5th edition published in 2010 to the 9th edition published in 2023 [2, 6–9]. The definitions of the categories and grading system are summarized in Table 1.

We compared the total number of categories and grades across the fifth through ninth editions. Additionally, to understand the proportion of each category and grade, we investigated changes in the number of fact sheets and indications for each edition.

Next, we individually compared each indication to assess the evolution of the evidence. We compared the category and grade of indications in the ninth edition with those at their initial introduction in the guidelines. For example, indications that were first included in the guidelines were compared with their corresponding values in the ninth edition. Similarly, the category and grade of indications newly introduced in the seventh edition were compared with the values in the ninth edition.

TABLE 1 Category and grade recommendation definitions for therapeutic apheresis.

	Description
Category	
I	Disorders for which apheresis is accepted as first-line therapy, either as a primary stand-alone treatment or in conjunction with other modes of treatment.
II	Disorders for which apheresis is accepted as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment.
III	Optimum role of apheresis therapy is not established. Decision-making should be individualized.
IV	Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful. IRB/Ethics Committee approval is desirable if apheresis treatment is undertaken in these circumstances.
Recommendation	
Grade 1A	Strong recommendation, high-quality evidence
Grade 1B	Strong recommendation, moderate-quality evidence
Grade 1C	Strong recommendation, low-quality or very low-quality evidence
Grade 2A	Weak recommendation, high-quality evidence
Grade 2B	Weak recommendation, moderate-quality evidence
Grade 2C	Weak recommendation, low-quality or very low-quality evidence

Abbreviation: IRB, Institutional Review Board.

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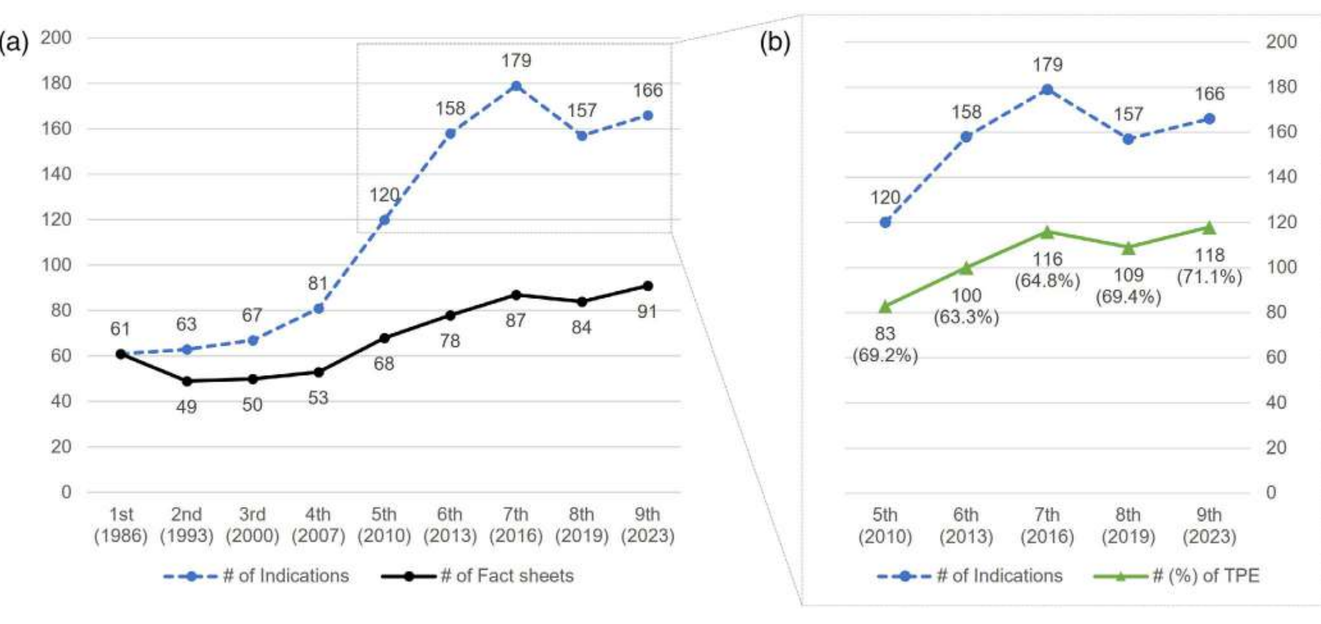

FIGURE 1 (a) Number of fact sheets and indications from the first through the ninth edition. (b) Number of total indications and number (%) of indications of therapeutic plasma exchange (TPE) from the fifth to the ninth edition.

Grade changes from A to B or C, or from B to C, indicated a weakening in the quality of evidence. In contrast, changes from C to A or B, or from B to A, indicated an improvement in the quality of evidence, suggesting strong support for the indication.

In case of the classification of the disease, condition or indication changed, it was adjusted to fit the clinical context of the latest edition.

RESULTS

The ASFA ninth edition contained a total of 91 fact sheets and 166 indications. Since the publication of the first edition, the number of fact sheets and indications tended to increase. Compared with the previous edition in 2019, the number of fact sheets and indications increased by nine and seven, respectively (Figure 1a).

Of the 166 indications in the ninth edition, 118 indications (71.1%) were related to TPE, including indications where TPE was either the sole procedure or part of multiple modalities. From the fifth to ninth editions, indications related to TPE have consistently been the most frequent (Figure 1b).

The 118 indications related to TPE in the ninth edition were categorized as follows: 72 in Category III, 23 in Category II, 20 in Category I and 3 in Category IV. Of the indications, 86 were Grade 2 and 32 were Grade 1, indicating a 2.7 times higher prevalence of weak recommendation levels over strong ones. The breakdown by evidence level showed 75, 35 and 8 indications as level C, B and A, respectively. Higher quality evidence corresponded to fewer indications. Grade 2C was most common ($n = 64$), followed by Grades 2B, 1B, 1C, 1A and 2A.

Considering the category and grade together, the indication with III/2C accounted for 41.5%. For indications with III/2C, TPE cannot be considered as a first- or second-line therapy due to the weak evidence. Instead, individualized clinical decision-making is essential based on each patient's specific clinical condition. The categories and recommendation grades for TPE indications in the ninth ASFA guidelines are outlined in Figure 2.

In the fifth edition, all four categories showed no significant differences, primarily falling under Category III. However, from the sixth to the ninth edition, there was a noticeable increase in both the number and proportion of indications, reaching 61.0% in the ninth edition. Categories I and II each had around 20 indications, while Category IV steadily declined to only three indications by the ninth edition. Grade 2C consistently represented the highest proportion across all editions. The details are illustrated in Figure 3.

Throughout the four revisions, from the fifth to the ninth edition, the grades changed to a total of 24 TPE indications (Figure 4). Most indications underwent only one grade update. However, three indications underwent two revisions and one indication underwent three revisions.

However, the grading system combined both the recommendation and evidence levels. We reviewed indications based on changes in the evidence level (A–C), independent of any revisions to the recommendation (1 or 2). As a result, 16 indications changed their evidence level from the fifth to the ninth edition. Among them, 10 indications had upgraded from B to A or from C to A or B, indicating a strengthening of the evidence. For example, neurological indications such as acute attack/relapse of multiple sclerosis and neuromyelitis optical spectrum disorder have been updated from Grade B to A and from Grade C to B, respectively (Figure 4). The other 6 indications experienced downgrades in their evidence level, from A to B or from B to C, indicating a decrease in the strength of evidence (Figure 5).

Additionally, some indications for TPE either newly appeared or were removed in the ninth edition, and assessing changes in their

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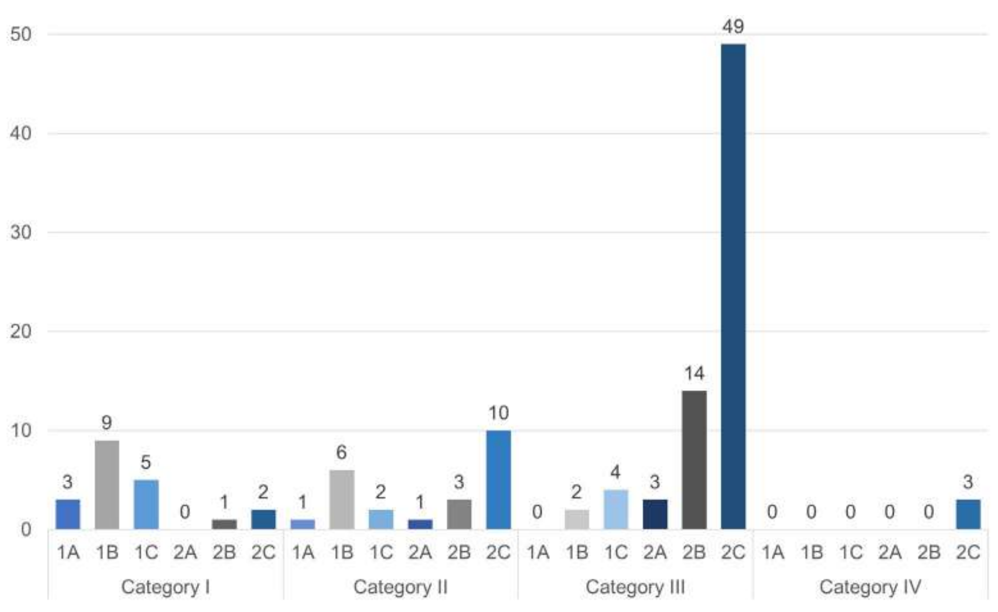
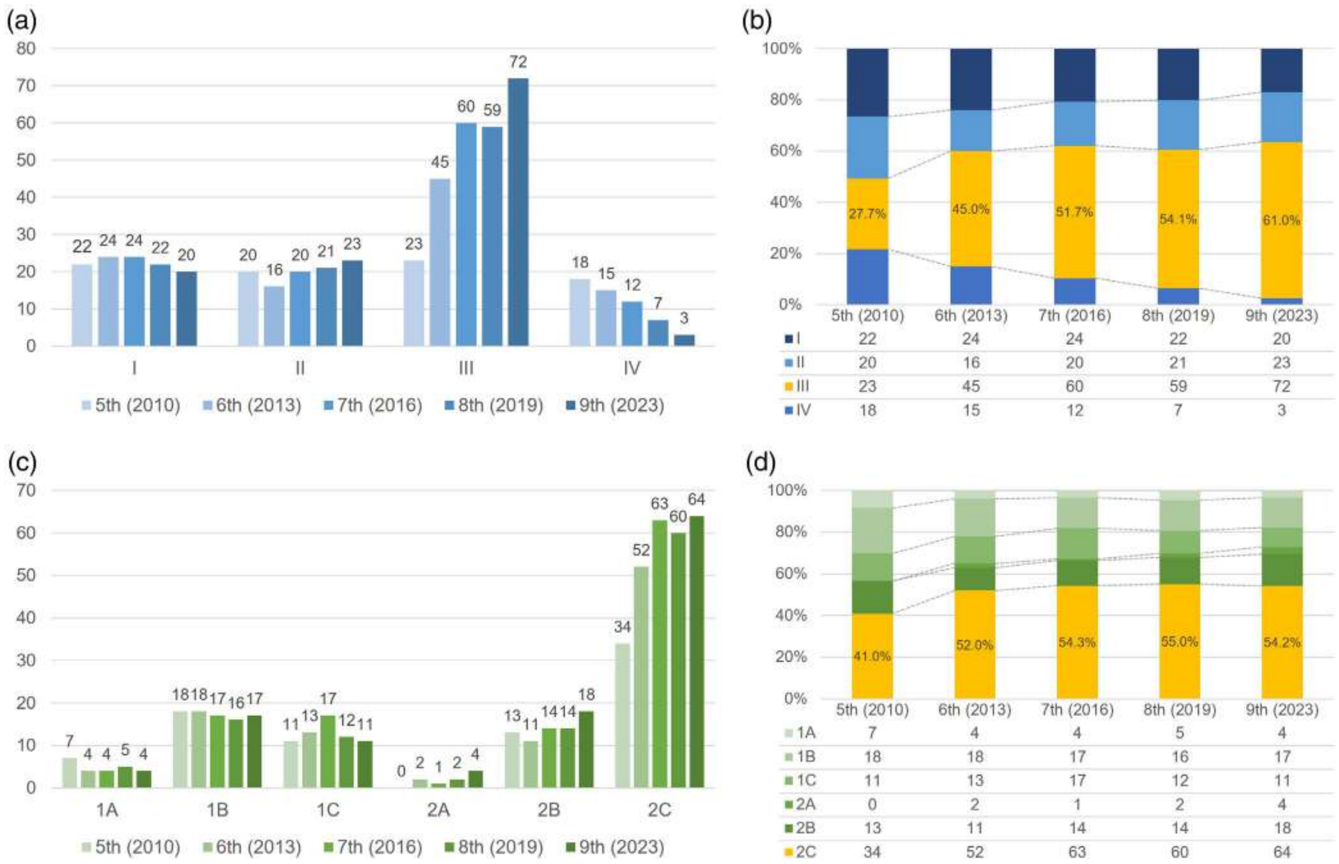


FIGURE 2 Category and grade distribution of therapeutic plasma exchange indications in the ninth edition.



	5th (2010)	6th (2013)	7th (2016)	8th (2019)	9th (2023)
I	22	24	24	22	20
II	20	16	20	21	23
III	23	45	60	59	72
IV	18	15	12	7	3

	5th (2010)	6th (2013)	7th (2016)	8th (2019)	9th (2023)
1A	7	4	4	5	4
1B	18	18	17	16	17
1C	11	13	17	12	11
2A	0	2	1	2	4
2B	13	11	14	14	18
2C	34	52	63	60	64

FIGURE 3 Category (a, b) and grade (c, d) distribution of therapeutic plasma exchange indications in the fifth through ninth editions.

category or grade was impossible. In the ninth edition, four Category IV indications in the previous edition and 18 indications were newly added for TPE. Retired or new indications for TPE are summarized in Table 2.

DISCUSSION

According to World Apheresis Association registry data, centrifugal plasma exchange is the most frequent apheresis procedure, accounting

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<table>
<tr><th></th><th>2010 (5th)</th><th>2013 (6th)</th><th>2016 (7th)</th><th>2019 (8th)</th><th>2023 (9th)</th></tr>
<tr><td>ANCA-associated RPGN, DAH</td><td>1B</td><td colspan="4">1C</td></tr>
<tr><td>Anti-glomerular basement membrane disease, Dialysis dependence, no DAH</td><td>1A</td><td colspan="4">2B</td></tr>
<tr><td>Anti-glomerular basement membrane disease, Dialysis independence</td><td>1A</td><td>1B</td><td>1C</td><td colspan="2">1B</td></tr>
<tr><td>Atopic dermatitis, recalcitrant</td><td></td><td></td><td colspan="2">2C</td><td>2B</td></tr>
<tr><td>Chronic acquired demyelinating polyneuropathies, IgM</td><td colspan="3">1C</td><td colspan="2">1B</td></tr>
<tr><td>Cryoglobulinemia, Severe/symptomatic</td><td>1B</td><td colspan="4">2A</td></tr>
<tr><td>Familial hypercholesterolemia, All patients</td><td colspan="3">1C</td><td colspan="2">1B</td></tr>
<tr><td>Focal segmental glomerulosclerosis, Recurrent in kidney transplant</td><td>1C</td><td colspan="4">1B</td></tr>
<tr><td>Hypertriglyceridemic pancreatitis, Severe</td><td colspan="3">2C</td><td colspan="2">1C</td></tr>
<tr><td>Immune thrombocytopenia, Refractory</td><td>1C</td><td colspan="4">2C</td></tr>
<tr><td>Multiple sclerosis, Acute attack/relapse</td><td colspan="3">1B</td><td colspan="2">1A</td></tr>
<tr><td>Neuromyelitis optical spectrum disorder, Acute attack/relapse</td><td>1C</td><td colspan="4">1B</td></tr>
<tr><td>Neuromyelitis optical spectrum disorder, Maintenance</td><td>1C</td><td colspan="4">2C</td></tr>
<tr><td>Pediatric autoimmune neuropsychiatric disorders, Sydenham's chorea, severe</td><td colspan="2">1B</td><td colspan="3">2B</td></tr>
<tr><td>Pemphigus vulgaris, Severe</td><td>2B</td><td>2C</td><td colspan="3">2B</td></tr>
<tr><td>Psoriasis</td><td>1B</td><td colspan="4">2C</td></tr>
<tr><td>Sepsis with multiorgan failure</td><td colspan="4">2B</td><td>2A</td></tr>
<tr><td>Sudden sensorineural hearing loss</td><td></td><td colspan="2">2C</td><td colspan="2">2A</td></tr>
<tr><td>Thrombotic microangiopathy, drug induced, Quinine</td><td>2B</td><td colspan="4">2C</td></tr>
<tr><td>Thrombotic microangiopathy, drug induced, Ticlopidine</td><td>2B</td><td>1B</td><td colspan="3">2B</td></tr>
<tr><td>Transplantation, heart, Desensitization</td><td></td><td>2C</td><td colspan="3">1C</td></tr>
<tr><td>Transplantation, liver, Desensitization, ABOi, living donor</td><td>2C</td><td colspan="4">1C</td></tr>
<tr><td>Vasculitis, ANCA associated, Eosinophilic granulomatosis with polyangiitis</td><td></td><td></td><td>1B</td><td colspan="2">2C</td></tr>
<tr><td>Voltage-gated potassium channel antibody-related diseases</td><td></td><td>1C</td><td>2C</td><td colspan="2">1B</td></tr>
</table>

Color index: 2C 2B 2A 1C 1B 1A

FIGURE 4 Grade trends demonstrating 24 indications with grade updates in the fifth through ninth editions. ANCA, antineutrophil cytoplasmic antibody; DAH, diffuse alveolar haemorrhage; IgM, immunoglobulin M; RPGN, rapidly progressive glomerulonephritis.

for 28% of all procedures conducted and 64.0% of procedures after excluding preparative procedures [10]. In addition, TPE has been the predominant procedure in the ASFA guidelines, representing the highest proportion of procedures (71.1%) in ninth edition. All other cases of retired or newly introduced fact sheets and indications have been attributed to TPE, with only one indication of extracorporeal photopheresis. Moreover, TPE is well-suited for evaluating an evidence-based approach; among the various apheresis procedures, TPE is the most widely used, resulting in a higher number of published literatures compared with other procedures. In addition, TPE is the most prevalent procedure (92.4%) in Korea [11], while other procedures such as adsorptive cytapheresis, immunoadsorption, lipoprotein apheresis or double filtration plasmapheresis are rarely performed.

Since the fifth edition, only five to eight indications had strong evidence corresponding to Grades 1A and 2A. In contrast, Grade 2C indications consistently represented the highest percentage compared with other grades each year. Newly introduced indications were mostly classified as Grade 2C, which might have influenced the high frequency of Grade 2C indications. Although weak evidence predominated overall, indications with a strengthened evidence quality outnumbered those with a weakened evidence quality throughout the last four revisions.

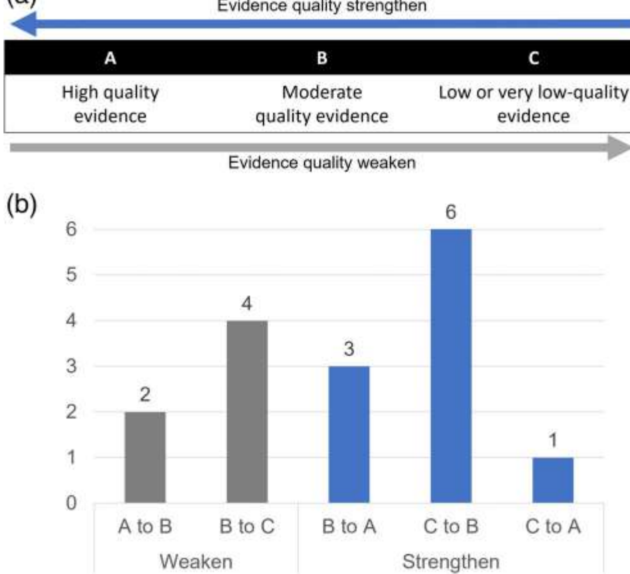

FIGURE 5 Comparison of the evidence quality in the fifth through ninth editions. (a) Summary of the level of evidence from American Society for Apheresis guidelines. (b) The number of indications with weakened or strengthened evidence.

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TABLE 2 Retired Category IV recommendations and newly introduced fact sheets and indications in 2023.

Disease/condition	Indication	Procedure	Category	Grade
Retired categories				
Amyloidosis	Causes other than dialysis	TPE	IV	2C
HELLP syndrome	Antepartum	TPE	IV	2C
Vasculitis, other	Idiopathic polyarteritis nodosa	TPE	IV	1B
Paraproteinemic demyelinating neuropathies; chronic acquired demyelinating polyneuropathies	Multifocal motor neuropathy	TPE	IV	1C
New fact sheets				
Alzheimer's disease	Mild or moderate	TPE	III	2A
Autoimmune dysautonomia		TPE	III	2C
Idiopathic inflammatory myopathies	Anti-synthetase syndrome	TPE	III	2B
	Clinically amyopathic dermatomyositis	TPE	III	2B
	Immune-mediated necrotizing myopathies	TPE	III	2B
Immune checkpoint inhibitors, immune-related adverse events		TPE	III	2C
Paraneoplastic autoimmune retinopathies		TPE	III	2C
Transplantation, intestine	Antibody-mediated rejection	TPE	III	2C
	Desensitization	TPE	III	2C
Vaccine-induced immune thrombotic thrombocytopenia	Refractory	TPE	III	2C
New indications incorporated into existing fact sheets				
Acute liver failure	Acute fatty liver of pregnancy	TPE	III	2B
Acute toxins, venoms and poisons	Other[a]	TPE/RBC exchange	III	2C
Sickle cell disease, acute	Other complications[b]	RBC exchange/ TPE	III	2C
Chronic acquired demyelinating polyneuropathies	CANOMAD/CANDA	TPE	III	2C
Thrombotic microangiopathy, pregnancy associated	Extremely preterm preeclampsia, severe	TPE/LA	III	2C
Transplantation, heart	Rejection prophylaxis	TPE	II	1C
Vasculitis, other	Kawasaki disease	TPE	III	2C
	Multisystem inflammatory syndrome in children	TPE	III	2C

Abbreviations: CANDA, chronic ataxic neuropathy with disialosyl antibodies syndrome; CANOMAD, chronic ataxic neuropathy, ophthalmoplegia, immunoglobulin M paraprotein, cold agglutinins and disialosyl antibodies syndrome; HELLP, haemolysis, elevated liver enzymes, low platelets; LA, lipoprotein apheresis; RBC, red blood cell; TPE, therapeutic plasma exchange.
[a]Methemoglobinemia incorporated into acute toxins, venoms and poisons.
[b]Bone marrow necrosis/fat embolism syndrome incorporated into sickle cell disease.

The combination of Category III and Grade 2C indications appeared most frequently, which could have several explanations. The primary reason was the limited use of TA procedures, which complicated the recruitment of a significant number of study participants. The challenge in conducting large-scale studies is not only due to the rarity of a clinical status requiring TPE but also the fact that many TPE procedures are performed for acute situations or emergencies. Furthermore, in many cases, TPE is combined with other treatments, such as intravenous immunoglobulin, rather than being the only treatment, which makes it difficult to assess its effectiveness. Additionally, the high prevalence of Category III indications implies that the decision to perform TA is highly dependent on the clinical status of the individual patient. Given these considerations, conducting large-scale randomized controlled trials and obtaining strong evidence can be exceedingly challenging. This can also be confirmed by the fact that Grades 1A and 2A occur the least frequently in each edition compared with other grades (Figure 3c).

Despite being the most extensively used guidelines worldwide, international healthcare workers who refer to the ASFA guidelines must be aware of regional limitations. Although the composition of the Journal of Clinical Apheresis Special Issue Writing Committee member is intended to have a wide range of geographical and clinical backgrounds, it is still limited to North American and European countries [2]. Certain diseases and conditions requiring TA as a key treatment have a very low prevalence in the Writing Committee's designated region of interest and are not covered by the guidelines. One notable example is severe fever with thrombocytopenia

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syndrome (SFTS). In Korea, SFTS has a relatively high prevalence and numerous case reports, and studies regarding the use of TPE have been reported [12–14]. However, due to the low prevalence in North America, SFTS is not included in ASFA guidelines. Moreover, the language barriers due to the ASFA guidelines' focus on English-written literature might result in non-English literature not being adopted as evidence. While the membership of ASFA was queried for potential new indications before the revision of the guidelines, international collaboration with global apheresis community would help to make a more comprehensive review.

Furthermore, an evidence-based approach is inherently dependent on the published literature available. The current criteria for including guidelines usually require at least 10 documented cases in the previous decade. Considering that scenarios involving rare conditions that occur infrequently are not conducive to extensive case series or randomized controlled trials, it should be considered whether researchers are allowed to submit additional case reports when only nine case reports have emerged over the past 10 years, specifically within the realm of English publications. For example, pancreatic transplantation has been considered a candidate since the seventh edition in 2016 [2, 8, 9] but remains a candidate in the ninth edition due to insufficient evidence. Experts in apheresis should actively engage in research and publish papers on indications with infrequent procedures but significant therapeutic effects. And the efforts will serve as the evidence for inclusion in the guidelines for future. To overcome the regional and frequency challenges of indications, collaborative studies such as international or multi-institutional research will be essential.

In summary, most indications are assessed as Category III and Grade 2C, and new indications are likely to be classified as III/2C. Therefore, until more evidence accumulates, decisions for indications with a low grade should be based on each patient's clinical status, and the role of experts is crucial.

In conclusion, the quality of evidence has improved with each revision; however, larger scale studies are still needed. To make progress, conducting nationwide and international studies is important.

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H.J.K. collected and organized data and wrote the initial draft of the manuscript, Y.C. and H.K. analysed data and reviewed the manuscript, S.-H.H. and H.-B.O. reviewed and edited the manuscript and D.-H.K. conceptualized and supervised this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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ORIGINAL ARTICLE

Haemolytic transfusion reactions caused by non-ABO red cell antibodies reported to the Norwegian Haemovigilance System 2004–2020

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Abstract

Background and Objectives: The aim of this study was to analyse the reports received in the Norwegian Haemovigilance System from 2004 to 2020 on acute and delayed haemolytic transfusion reactions caused by non-ABO red cell antibodies.

Materials and Methods: Antibody specificity, clinical symptoms and outcomes were included when available.

Results: After transfusion of 3.7 million red cell concentrates, reports on 78 cases of haemolytic transfusion reactions caused by non-ABO red cell antibodies were received, corresponding to an incidence of 1 in 47,000 transfused red cell concentrates. There were 30 acute and 48 delayed haemolytic transfusion reactions. A total of 113 red cell antibodies were found: 82 alloantibodies, 6 autoantibodies and 25 cases where the antibody specificity could not be determined. Two fatalities occurred: one caused by anti-Wr^a and one caused by an unidentified red cell antibody. The most frequently reported antibody specificities were those in the Rh and Kidd blood group systems, representing 24% and 14%, respectively, of all the antibodies identified. In six cases, errors occurred, leading to the issuing of blood units without the required phenotype match.

Conclusions: Despite the possible underreporting, the low number of serious haemolytic transfusion reactions reflects an adequate pre-transfusion practice by the Norwegian blood banks.

Keywords

antibody specificity, haemolytic transfusion reaction, haemovigilance, red cell antibody, serological investigations

Highlights

- The overall risk for experiencing a haemolytic transfusion reaction (HTR) caused by non-ABO antibodies is 1 per 47,000 red blood cell (RBC) transfusions.
- The most frequently identified antibody specificities were those in the Rh and Kidd blood group systems. In 32% of all reports, the specificity of the antibody/ies could not be determined.

We present data received in the Norwegian Haemovigilance Working Group in the period 2004–2020, regarding acute and delayed transfusion reactions caused by non-ABO red cell antibodies.

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- In 26% of all HTRs, errors regarding the selection of RBCs led to the transfusion of units that did not comply with the antigen requirements according to the patient's records on alloimmunization.

INTRODUCTION

The Norwegian Haemovigilance System was implemented in 2004 as a voluntary reporting and learning system, becoming mandatory in 2007 [1]. Transfusion reactions, blood donor complications and near misses are reported electronically. All reports are validated by the Norwegian Haemovigilance Working Group before inclusion in the database and the annual reports. Serological reactions with no symptoms of haemolytic transfusion reactions (HTRs) are not reported.

The definition of HTR in use in Norway is that proposed by the International Society of Blood Transfusion Working Party on Haemovigilance [2]. Briefly, an acute haemolytic reaction is defined as having one or more symptoms such as fever, chest and/or back pain and hypotension and/or laboratory parameters consistent with haemolysis within 24 h after a transfusion. When similar symptoms occur between 24 h and 28 days after a transfusion, the reaction is regarded as a delayed transfusion reaction.

In Norway, the 'Type & Screen' (T&S) approach for issuing red blood cells (RBCs) has been in use for many years. T&S results are valid for 4 days, regardless of whether the patient is alloimmunized or not, or if the patient has received a transfusion within the 4 days since the last T&S was performed. For patients with no known red cell antibodies, the blood unit may be issued by electronic crossmatch. For patients with past or present red cell antibodies, the antiglobulin crossmatch must always be performed before the transfusion, and the result of a negative crossmatch is valid for 4 days. Transfusion records of previous alloimmunization cannot be automatically accessed across blood banks due to legal restrictions, although in special situations, this information may be requested.

Extended antigen matching for Rh and Kidd blood group systems to reduce the risk of alloimmunization is recommended for certain patient groups, such as patients with haemoglobinopathies, haematological malignancies and/or red cell autoantibodies.

RhD-negative patients with childbearing potential receive RhD-negative RBCs. All patients with childbearing potential should receive K-negative RBCs. There are no other national guidelines for antigen requirements in this patient group, even if some blood banks would avoid giving Rhc-positive blood units to an Rhc-negative patient with childbearing potential, as anti-c alloimmunization may lead to serious complications in pregnancy. We assume that the recommendations for extended antigen matching are followed as far as the blood bank inventory allows it, but we do not have data to confirm this.

In Norway, blood banks are hospital-based, and 47 of them perform routine pre-transfusion tests. Only 24 blood banks do antibody identification. Blood banks that do not perform antibody identification send the blood samples to their local reference laboratory in immunohaematology for further investigations. The Norwegian National Reference Laboratory on Immunohaematology at Oslo University Hospital has been responsible for the National Quality Assessment since 1994, and all Norwegian blood banks participate. The programme includes ABO/RhD typing, phenotyping, crossmatch, direct antiglobulin test (DAT), red cell antibody screening and identification, as well as antibody titration. In addition, once a year, a hypothetical serological case is included for discussion, and the participants may explain which investigations should be performed. Norwegian blood banks appears to perform adequately, as shown in the results of the Norwegian Quality Assessment Program in Immunohaematology (no official report available). Many blood banks also participate in international quality assessment programmes.

An HTR is usually suspected by the nurse in charge of the transfusion when the patient experiences a change in the clinical signs and symptoms compatible with haemolysis under or within days after a red blood cell transfusion. Underreporting cannot be excluded, since many HTRs may be mild or subclinical and therefore not reported to the blood bank by the clinicians. In many cases, symptoms such as fever or changes in the blood pressure may be attributed to the patient's underlying medical condition rather than to an HTR. When an HTR is reported to the blood bank, a serological investigation is performed, both in the pre-transfusion sample and in the sample taken after the transfusion reaction.

There are national recommendations regarding serological investigations after an HTR, which should be performed both in blood samples taken before and after the transfusion reaction. These tests include ABO/RhD typing of the patient and the blood unit, as well as red cell antibody screening and DAT in the patient blood samples, and immediate spin and antiglobulin crossmatch [3]. Additional phenotyping of the blood unit may be necessary. When there are discrepancies in the results before and after the transfusion reaction, or in case new antibodies are identified, an HTR should be suspected and further serological investigations must be performed.

MATERIALS AND METHODS

Haemovigilance reports on acute and delayed HTRs caused by non-ABO red cell antibodies received between 2004 and 2020 were included. The specificity, nature (both alloantibodies and autoantibodies) and number of red cell antibodies identified in patients reported to have an HTR were included. The clinical symptoms, clinical outcome, immunohaematological investigations, additional laboratory test results and information regarding cases of documented alloimmunization prior to the HTR were provided in many reports. We only present the results of the antibody specificities suspected to be related to the HTR. Antibodies identified before transfusion, leading

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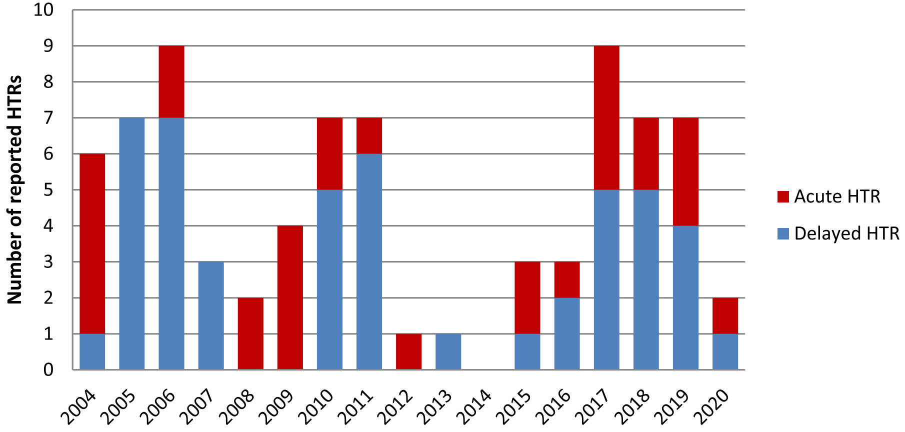

FIGURE 1 Annual distribution of haemovigilance reports for acute and delayed haemolytic transfusion reactions (HTRs) received in the period 2004–2020.

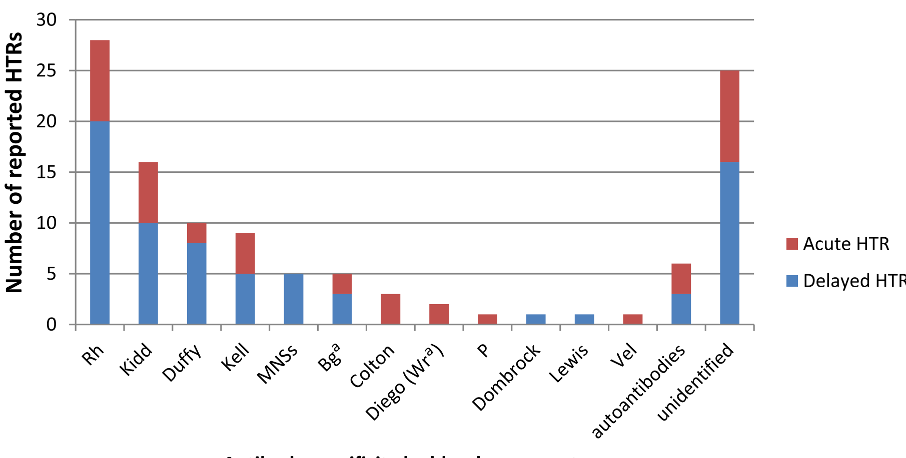

FIGURE 2 Distribution of the reported cases of acute or delayed haemolytic transfusion reactions (HTRs) by blood group system of the red cell antibodies suspected to have been the cause of the reaction.

to the use of blood negative for the corresponding antigen, are not included. HTRs caused by ABO incompatible transfusions are reported as a distinct category to the Norwegian Haemovigilance System and are outside the scope of this study.

RESULTS

We received reports on transfusion reactions from all the blood banks in Norway. The number of reports corresponded with the size of the hospital and the number of transfusions.

From 2004 to 2020, approximately 3.7 million RBCs were transfused in Norway. In this period, 78 cases of HTRs caused by non-ABO red cell antibodies (30 acute and 48 delayed HTRs) were reported (Figure 1). This corresponds to an overall risk of 1 HTR per 47,000 RBCs transfused (1 acute HTR per 123,000 RBCs transfused and 1 delayed HTR per 77,000 RBCs transfused). A total of 113 red cell antibodies were involved in the HTRs, consisting of 82 alloantibodies, 6 autoantibodies and 25 cases where the antibody specificity could not be determined. Two fatalities occurred: one caused by anti-Wr^a [4] and one caused by an unidentified red cell antibody.

Antibody specificities identified in the HTRs

The most frequently reported antibody specificities were those in the Rh and Kidd blood group systems, with 28 and 16 reports, respectively, representing 25% and 14% of all antibodies, respectively (Figure 2). Anti-Jk^a and anti-E were the most frequently identified antibody specificities, with 12 reports each (Figure 3). Anti-E was identified in 12 reports, wherein 10 cases as the only antibody specificity. Anti-Jk^a was found as the only specificity in 11 out of the 12 reports where anti-Jk^a was identified (Figure 4).

Multiple alloantibodies were identified in 19 cases (24% of all the reports) in the serological investigations performed after the HTR, in 7 acute and 12 delayed HTRs. In seven cases with multiple antibodies,

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FIGURE 3 Distribution of the reported cases of acute and delayed haemolytic transfusion reactions (HTRs) by the specificities of the red cell antibodies suspected to have been the cause of the reaction.

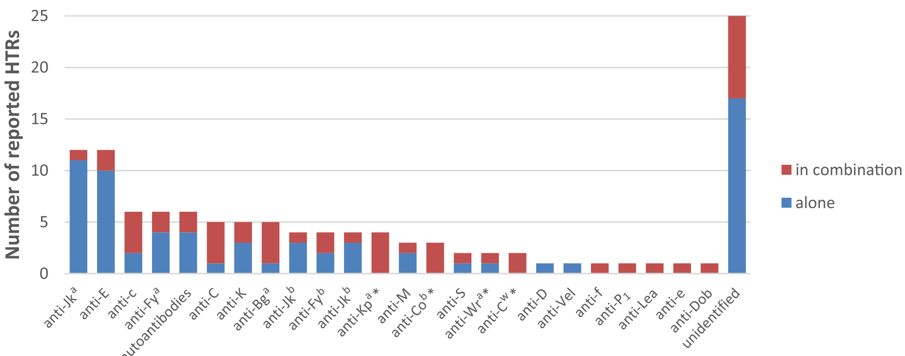

FIGURE 4 Frequencies of the red cell antibodies identified alone or in combination with other identified antibodies. *LIAs, antibodies against low-incidence antigens.

the specificity of one or more of the antibodies present in the patient's plasma could not be determined.

In seven cases (three anti-C, one anti-E, one anti-e and two anti-Jk^a), the alloantibody suspected of having been the cause of the HTR could only be identified by sensitive techniques such as PEG IAT (polyethylene glycol indirect antiglobulin test) and/or enzyme techniques, which are not routinely performed in the pre-transfusion testing.

Eleven red cell antibodies against low-incidence antigens (LIAs) not routinely present on the screening red cells, except for C^w, were identified. These were four cases of anti-Kp^a, two cases of anti-Wr^a, three cases of anti-Co^b and two anti-C^w (Figure 3). Except for one case of anti-Wr^a leading to a fatal acute HTR [4], the antibodies against LIAs were identified together with other antibody specificities. In two cases, two antibodies against LIAs could be found in the same patient, in addition to other red cell antibodies. In five other cases, one antibody specificity against LIAs was present, but other antibody specificities, such as anti-E, anti-P$_1$, anti-Fy^a, anti-Jk^a and others, were suspected to be the main cause of the HTR by the reporting blood bank.

In 12 cases, antibody specificities that usually have little clinical significance were reported as the suspected cause of 3 acute and 10 delayed HTRs. These were three anti-M, two anti-S, one anti-P$_1$, one anti-Le^a and five anti-Bg^a. In the two cases of acute HTR, anti-Bg^a was identified together with other alloantibodies of clinical significance (anti-c, anti-E, anti-Co^b and a possible anti-C). In four cases of delayed HTR (one anti-Bg^a, two anti-M and one anti-S), there were no other additional antibodies identified (Figure 4). One anti-P1 was highly suspected of having been the cause of the acute HTR, as the patient received a P$_1$-positive blood unit. The patient had also anti-Kp^a and anti-K, but the unit was negative for both Kp^a and K antigens.

In 25 cases (32% of all the reports), 9 acute and 16 delayed, the antibody specificity could not be determined.

In six cases when urgent transfusion was required, the blood unit was issued after a negative crossmatch but before the routine antibody screening test was completed. In five of these cases, the blood unit turned out to be positive for the red cell antigen the patient was immunized against, but the antibody was not reactive in the antiglobulin crossmatch, giving a false-negative result. We do not have information regarding the homozygosity or heterozygosity of the red cells used in the crossmatch.

Antibody specificities identified in the acute HTRs

We received 30 acute HTRs: 8 Rh antibodies, 6 Kidd antibodies, 5 antibodies against LIAs, 1 anti-P$_1$, 3 autoantibodies and

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9 unidentified antibodies. Figures 3 and 4 show a more detailed overview of the antibody specificities leading to an acute HTR in each blood group system.

There was one fatal case of anti-Wr^a. Anti-Co^b was identified in three cases of acute HTR, leading to a serious reaction.

For antibodies in the Rh system, anti-C was most frequently involved in acute HTRs, with four cases, followed by anti-E (two reports), anti-c (one report) and anti-e (one report). Anti-C was usually identified together with other antibody specificities. Only in one report, anti-C was found as the only antibody in a patient with symptoms of acute HTR (Figure 4). There was one case of acute HTR caused by anti-c. Two cases of anti-C, one anti-E and one anti-e could only be identified by using by sensitive techniques such as PEG-IAT and/or enzyme techniques.

In the Kidd system, anti-Jk^a was identified in four acute HTRs, followed by anti-Jk^b in two reports. Only in two cases of acute HTR, antibodies in the Duffy blood group system were identified (Figures 3 and 4). One anti-P_1 was highly suspected as to have been the cause of an acute HTR, as the patient received a P_1-positive blood unit.

Multiple alloantibodies were identified in seven cases leading to an acute HTR, where specificities against LIAs were present in five cases.

Red cell autoantibodies were identified in three acute HTRs, where no additional antibody specificities could be found (Figures 3 and 4).

Antibodies against LIAs were suspected to have been the cause of five acute HTRs (two anti-Wr^a and three anti-Co^b) (Figures 2 and 3).

Antibody specificities identified in the delayed HTRs

In 48 cases, the patient experienced a delayed HTR. As for acute HTRs, Rh and Kidd antibodies were also the most frequently reported specificities identified in delayed HTRs, with 20 and 10 cases, respectively (Figures 3 and 4). There were five reports on delayed HTR caused by anti-c. One anti-C and two anti-Jk^a, found in three delayed HTRs, could only be identified by using sensitive techniques.

Red cell autoantibodies were identified in one report on delayed HTR with no accompanying alloantibodies. In 16 cases of delayed HTR, the antibody specificity/ies could not be determined. Figures 3 and 4 show a more detailed overview of the antibody specificities leading to a delayed HTR in each blood group system.

Multiple alloantibodies were identified in 12 cases of delayed HTRs, where antibodies against LIAs were identified in two of these cases.

Avoidable HTRs

In 20 cases (26% of all HTRs), the patient was alloimmunized prior to the HTR, and the antibody specificities were known prior to the transfusion, but the blood units did not comply with the antigen requirements according to the patients' alloimmunization. These HTRs could therefore have been avoided. The errors made when issuing the blood units were attributed to stress situations caused by the urgent need for transfusion, that standard procedures were not followed, wrong interpretation of the crossmatch results, practical reasons regarding transfusion to outpatients and/or inadequate data registration in the laboratory information management system (LIMS). Even if all blood banks in Norway have LIMS, the use of warnings is not standardized, and they may be misunderstood or overlooked.

Reported clinical symptoms and laboratory findings related to the HTRs

In 42 cases (23 of the 30 cases of acute HTRs and 19 of the 48 delayed HTRs), information on clinical symptoms or relevant laboratory parameters was provided in the report. Fever and/or chills, alone or with other clinical symptoms, were reported in 18 cases, corresponding to 23% of all the total number of reports, whereas gastrointestinal (GI) symptoms such as nausea and vomiting were described in 11 cases, corresponding to 14% of the reports on HTR. Icterus was reported in six cases, together with other signs of haemolysis.

In 35 cases (16 acute and 19 delayed HTRs), clinical symptoms and/or laboratory findings compatible with haemolysis were provided in the haemovigilance report. Icterus, back pain, Hb fall, haemoglobinuria, haemolysis in the post-transfusion blood sample, reduced haptoglobin, increased bilirubin and/or lack of Hb rise after the transfusion were reported. GI symptoms such as nausea, abdominal pain and diarrhoea were reported together with other symptoms or laboratory findings in 11 cases (14% of all reports). Unfortunately, we have limited information on other clinical symptoms and signs.

Anti-Co^b was identified in three cases of acute HTR, where two patients experienced back pain, and the third patient had oliguria. One of these patients had a transient renal failure requiring haemodialysis, but the patient made a full recovery.

In nine additional cases involving anti-Jk^a, anti-C, anti-c, anti-Fy^a, anti-Fy^b, one unidentified antibody, anti-Jk^b, autoantibodies and anti-E, the patient experienced chest or back pain and/or signs of haemolysis during the HTR. In one fatal case of anti-Wr^a, the patient had severe renal failure [4].

DISCUSSION

In this overview, we present the findings related to haemovigilance reports on acute and delayed HTRs caused by non-ABO antibodies. The number of reports on HTRs has been stable, with one to nine reports per year. Underreporting cannot be excluded, since many HTRs may be mild or subclinical and therefore not reported to the blood bank by the clinicians. In many cases, symptoms such as fever or changes in the blood pressure may be attributed to the patient's underlying medical condition rather than to an HTR.

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In our haemovigilance material, Kidd and Rh antibodies were the most frequent specificities identified in the reported HTRs. This is in accordance with data from the SHOT report 2022, which showed that anti-Jk[a] remains the most common antibody implicated in delayed HTRs [5]. Antibodies in the Kidd blood group system can be difficult to identify as they tend to become weaker over time, but even weak antibodies in the Kidd system can be capable of causing HTRs [6]. Our data show that even weakly reactive antibodies in the Rh and Kidd blood group systems may lead to an acute or delayed HTR. This is comparable to the results of the SHOT report for 2022, where 9 of the 11 antibodies related to an acute HTR as well as 21 of the 29 cases of delayed HTR were identified only in the post-transfusion sample [5]. The use of more sensitive techniques for the identification of weakly reactive red cell antibodies, such as PEG-IAT and enzyme techniques, has been shown to be useful in reducing the risk of HTRs [7]. These measures may, however, have detrimental effects by delaying a transfusion, as they may lead to false-positive reactions or detect weak antibodies with little clinical significance. The use of more sensitive serological techniques cannot prevent HTRs caused by antibodies against LIAs.

In four cases, antibodies against LIAs were involved in a serious HTR. Even if there have been several reports of severe or fatal HTRs caused by antibodies against LIAs [8], it is not recommended to include red cells positive for LIAs routinely in the screening and/or identification panels [9, 10].

In 12 cases, the HTR was attributed to red cell antibodies that usually have little clinical significance, such as anti-M, anti-S, anti-P_1, anti-Le[a] and anti-Bg[a]. There are only sporadic reports where strongly reactive Bg[a] antibodies were involved in an HTR [11, 12]. It is difficult to assess whether these antibodies were the cause of the reaction or if other, non-identified antibodies might also have been present. In addition, when multiple red cell antibodies are involved in an HTR, such as in the two cases of acute HTR where anti-Bg[a] was identified, it is difficult to assess the causative antibody specificity, as we do not usually have detailed information about the phenotype of the transfused blood units involved in the HTR.

In 32% of all reports, the specificity of the antibody/ies could not be determined. Extensive investigations after a serious HTR should be performed in order to try to identify the causative red cell antibody/ies. We strongly recommend the referral of blood samples after serious transfusion reactions highly suspected to be an HTR to a reference laboratory, when the initial antibody identification is inconclusive at the local blood bank. In many cases, the use of special serological techniques, such as differential absorption, antibody elution, red cell antigen genotyping and use of rare red cell panels and antisera may be necessary. We also encourage the reporting blood bank to provide as much information as possible in the haemovigilance report on serological findings, other relevant laboratory results as well as clinical symptoms and patient outcome. In cases were additional information is requested, it is often too late to perform additional tests.

In 14% of the reports, GI symptoms such as nausea and vomiting were described in patients experiencing an HTR. GI symptoms are sometimes reported to the Norwegian Haemovigilance System as the only symptoms of a transfusion complication, and these reactions are considered as a non-specific transfusion reactions. Little is known regarding their pathophysiology, but based on our findings, GI symptoms should perhaps be suspected as a possible sign of the early stage of an HTR, as it has previously been suggested [13].

The overall incidence of alloimmunization in Norway is thought to be 0.57% in the pre-transfusion tests for possible recipients of blood [14]. It is estimated in the literature that only 30% of the present red cell antibodies are actually detected, due to alloantibody evanescence patterns, missed opportunities for alloantibody detection and record fragmentation [15]. Patients with previous history of red cell alloimmunization may be prone to developing additional antibodies [16], putting them at higher risk of experiencing an HTR. The use of extended antigen match in alloimmunized patients is not required in Norway, although it might be beneficial in patients with several antibody specificities and patients expected to have long-term need for transfusion [17]. For patients with warm autoantibodies, however, the use of prophylactic antigen match does not seem to have any protective effects for reducing the risk for new alloimmunization [18].

Urgent transfusions are sometimes required before full pre-transfusion and antibody investigations are completed, and this may lead to HTR if the patient is alloimmunized. This is a risk that clinicians need to be aware of when ordering emergency blood units. Clinicians should be able to promptly recognize the typical clinical symptoms and signs in patients experiencing an HTR.

In summary, in 17 years, we received 78 reports on HTRs corresponding to 1 HTR per 47,000 RBC transfusions. The most frequently identified antibody specificities were those in the Rh and Kidd blood group systems. In 26% of all HTRs, the patient was alloimmunized prior to the HTR, and the antibody specificity was known, but errors led to an HTR in the patient. Even if these HTRs could have been avoided, the low number of reported serious HTRs reflects that the blood banks in Norway have adequate strategies for antibody identification and selection of blood units. We strongly recommend the referral of blood samples to a reference laboratory when the initial antibody identification is inconclusive at the local blood bank.

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A.E. contributed to the original conception of the manuscript, performed the data analysis, elaborated the figures, reviewed the literature and wrote of the first draft of the manuscript; C.T.S. contributed to the review and editing of the manuscript; Ø. F. contributed to the review and editing of the manuscript. All the authors contributed to the revision of the manuscript and approved the submitted version.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ORIGINAL ARTICLE

Gender differences in scholarly productivity of early-career transfusion medicine physicians

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Abstract

Background and Objectives: Promotion in academic medicine requires evidence of the creation and dissemination of scholarly output, primarily through peer-reviewed publications. Studies demonstrate that scholarly activity and impact are lower for women physicians than for men physicians, especially during the early stages of their academic careers. This report reviewed physicians' academic productivity after passing their Blood Banking/Transfusion Medicine (BBTM) subspecialty exam to determine if gender discrepancies exist.

Methods: A cross-sectional analysis was designed to determine trends in scholarly activity for women physicians versus men physicians in BBTM. Indexed publications were reviewed using iCite, the National Institutes of Health (NIH) Office of Portfolio Analysis tool, from 1 January 2017 to 1 December 2021, for BBTM examinees who passed the sub-speciality fellowship exam in the years 2016 through 2018.

Results: Overall, women physicians had statistically significant fewer total career publications (median 6 vs. 9 cumulative papers, $p = 0.03$). Women published at a lower rate after passing BBTM boards, which was not statistically significant (0.7 vs. 1.3 publications per year). Other statistically significant findings include fewer early-career BBTM women physicians were first authors compared with men physicians ($p = 0.03$) and impact as assessed by relative citation ratio was higher for men ($p = 0.01$).

Conclusions: This study demonstrates that there are gender differences in scholarly productivity and impact on early-career BBTM physicians. Given that this cohort of BBTM physicians are early-career professionals, the significant difference in first authorship publications between women and men physicians is especially concerning. Publication metrics should be followed to ensure equitable research environments for early-career BBTM physicians.

Keywords

gender differences, scholarly productivity, transfusion medicine physicians

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Highlights

- Among transfusion medicine physicians, women had lower bibliometric scores in the first 3 years after passing the subspecialty fellowship examination compared with men.
- Similarly to other medical specialties, fewer women physicians in blood banking/transfusion medicine (BBTM) were attributed as first authors.
- Further studies into whether these disparities in early-career academic productivity hinder academic promotion and career advancement of women physicians in BBTM are warranted.

INTRODUCTION

Physicians, when compared by gender, are not equitably promoted to academic leadership positions in medicine [1]. The aetiology of this disparity in promotion is multi-factorial. Early-career achievement in scholarship could help combat this inequity, as could understanding the factors related to early-career academic rank promotion [2]. Competing demands can be identified for early-career academic physicians—including patient care responsibilities, education and scholarship—when junior faculty are at a vulnerable career point with a myriad of additional non-academic responsibilities such as child and elder care [3]. These factors have been shown to disproportionately impact women physicians more than men physicians [3].

Despite gender parity in many specialties in academic medicine (men and women at 50:50 ratios), several studies have shown discrepancies in scholastic bibliometric metrics of first author, senior author, corresponding author and citation-based analyses between genders [4–8]. For example, in ophthalmology, women physicians had lower academic productivity than men physicians and were less frequently identified in first- or senior-author positions [9]. These findings have been replicated in many medical specialties. However, the aetiology behind and the timing of when gender-based difference in academic scholarship/productivity emerges remains undetermined.

In a critical analysis of academic scholarship, Merman et al. [10] studied 28 medical specialties (excluding pathology) and highlighted that authorship of clinical practice guidelines underrepresented female physicians, the sex terminology used in the source publication. More recently, pathology-specific bibliometric scholarship analyses have shown similar gender inequities in academic productivity within clinical practice guidelines, surgical pathology book chapters, surgical pathology publications and pathology-based editorial board composition [11, 12]. Our prior work demonstrated that analysis of early-career cohorts within the pathology-based subspecialty of blood banking/transfusion medicine (BBTM) indicates considerable academic productivity; however, differences between genders in academic productivity among early-career BBTM physicians have not been studied to date [13].

To determine if there is a difference in BBTM early-career academic productivity by gender, we assessed bibliometric outputs over a 5-year period (2017–2021).

STUDY DESIGN AND METHODS

We performed subgroup analysis on our previously published cohort of physicians who passed the American Board of Pathology Transfusion Medicine/Blood Banking subspecialty board exam from 2016 to 2018 [13]. The group was assessed from 2017 to 2021. An a priori decision was made to assess academic output related to gender based on literature in other medical fields. Methods were based on a previously performed analysis characterizing the BBTM workforce [13]. The University of Texas Southwestern Human Research Protection Program reviewed the project and determined that the study did not require institutional review board approval or oversight as the information used in this analysis is within the public domain.

Early-career physicians are typically defined by medical associations as physicians within 10 years of graduation from their terminal fellowship programme or within 16 years of graduation from medical school [14]. We assessed the academic productivity of transfusion medicine physicians for 3 to 5 years of passing the boards.

Initial determination of gender was performed using the Gender API website, which determines the probability of societal gender based on name [15]. We used a threshold of >90% certainty for assignment. Gender was ultimately determined by consensus agreement among authors after a review of professional photos and available pronouns. There were no instances of they/them pronouns; all physicians were categorized as she/her or he/him.

Two independent data coders (TSI and BDA) were used to assess faculty ranking and authorship attribution. All discrepancies were evaluated by the research team. Individuals with common names whose total publication count was greater than 100 manuscripts were excluded in order to mitigate erroneous attribution of publications because such errors impact calculated metrics and thus skew results.

Publication data were obtained from the NIH iCite platform, a web-based portfolio tool that compiles author work and characterizes bibliographic metrics. Academic impact was assessed using the relative citation ratio (RCR), a metric that takes into account citations for similar contemporary works to normalize impact [16].

Statistical analyses were performed using GraphPad Prism (Version 9.2.0, GraphPad Software, San Diego, CA, USA). Normality was assessed using the Kolmogorov–Smirnov test. Descriptive statistics are reported as median values with interquartile ranges unless otherwise specified. Continuous variables were assessed using the Mann–Whitney test and categorical variables were

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assessed using the Fisher's exact test. Statistical significance was considered at p value <0.05.

RESULTS

A total of 133 individuals who passed the BBTM boards from 2016 to 2018 were included in the analyses. Five individuals were excluded due to having common names.

Demographics

The cohort consisted of 55% (70/128) men and 45% (58/128) women. The majority (66%, 85/128) of BBTM physicians worked in academia. Of these physicians, similar proportions of men, 64% (45/70), and women, 69% (40/58), worked within academic institutions. More men held additional degrees—such as MPH, MBA, or PhD—compared with women [31% (22/70) vs. 17% (10/58), $p = 0.07$]. Academic rank as assistant professor was relatively similar between genders. Eighty-four percent (38/45) of men were assistant professors, whereas 82.5% (33/40) of women held the same faculty rank. More women (17.5%, 7/40) were associate professors versus men 13.3% (6/45) ($p = 0.8$). Additional demographic data are presented in the previous publication [13].

Academic productivity by gender

A total of 1651 publications were produced by the 128 BBTM physicians. The median total career publications by men physicians was 9 (interquartile range [IQR]: 5–35), which was significantly higher than women physicians at 6 (IQR: 2–15; $p = 0.03$; Figure 1). Both genders in the BBTM cohort were academically productive since passing the boards. The median publication per individual per year since passing boards was 1.3 (IQR: 0.3–2.6) for men, whereas it was 0.7 (IQR: 0–2.1) for women ($p = 0.2$).

Authorship attribution by gender

Men physicians had a higher median number of publications as first authors than women physicians [3 (IQR 1–7.3) versus 2 (IQR: 0–5), respectively] ($p = 0.04$; Figure 2). There was no statistical difference in publications as middle author or senior author by gender.

Publication influence

The median total number of citations was higher for men compared with women [103 (IQR: 16.5–279.8) and 32.5 (IQR: 6.8–195), respectively], although this was not statistically significant ($p = 0.08$). Similarly, the median citations per paper were also higher for men [8.5 (IQR: 4.6–20.2)] compared with women [7.7 (IQR: 3.1–16.9)] ($p = 0.33$). The weighted RCR was significantly higher for men at 7.5 (IQR: 3.1–25.7) compared with 3.8 (IQR: 0.7–12.4) for women ($p = 0.01$; Figure 3).

DISCUSSION

Our study shows gender disparities in scholarly productivity and influence in early-career BBTM board-certified physicians. This is the first study to assess gender differences through the evaluation of bibliometric measures in peer-reviewed publications within BBTM. The median total number of publications by early-career BBTM women physicians was statistically significantly lower than that of men physicians. In addition, women were less frequently serving as first authors. The relative citation ratio which assesses scholarly impact was significantly higher for men BBTM physicians. These discrepancies

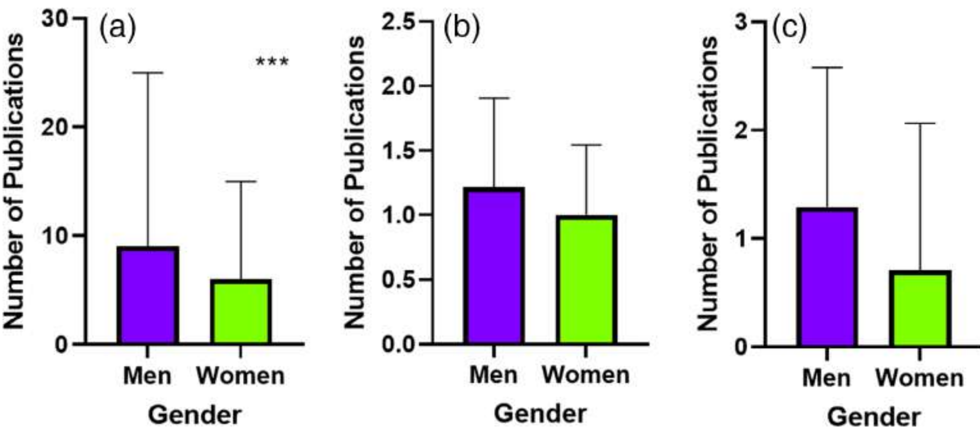

FIGURE 1 Publication data. (a) Cumulative publications ($p = 0.03$), (b) publications per year of academic activity ($p = 0.08$) and (c) publications per year since passing board exams ($p = 0.19$). ***Statistical significance.

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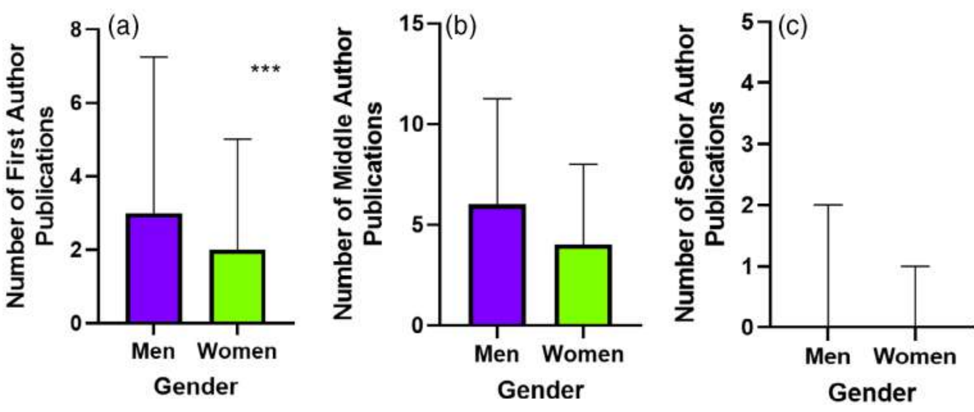

FIGURE 2 Authorship position. (a) first author publications ($p = 0.04$), (b) middle author publications ($p = 0.06$) and (c) senior author publications ($p = 0.13$). The median value for senior authorship was 0 for both groups. ***Statistical significance.

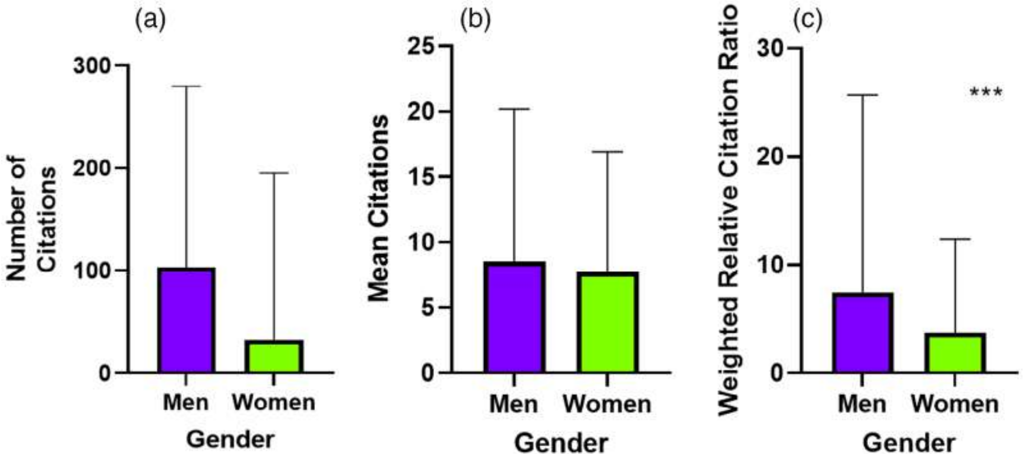

FIGURE 3 Citation characteristics. (a) total citations ($p = 0.08$), (b) mean citations per paper ($p = 0.33$) and (c) weighted relative citation ratio (RCR; $p = 0.01$). ***Statistical significance.

emphasize the potential influence of gender on the contribution, recognition and visibility of scholarly work by women (Table 1). These findings are consistent with results from other academic medical specialties.

The gender-based disparities identified in this study are consistent with previous studies in other academic medicine specialties. For example, a study on sex-based authorship trends, sex terminology used in source publication, in the highest-impact anaesthesiology journals over a 16-year period found that female physicians were less likely to be first and senior authors than male, despite increases in female representation in publications [17]. According to a 2013 publication, authorship attribution to females, sex terminology used in source publication, only represent 20% of author attribution in research publications [18]. Bibliometric analysis in specialties inside and outside pathology confirms gender imbalances in publication output and influence [5–7, 9, 11, 12, 17]. This is true despite the increasing number of women physicians entering academic medicine and a slightly higher proportion of women working in academia relative to men in our cohort.

Given that the majority of the women in our study worked in academia as assistant professors, the gender gap in academic productivity and publication influence may hinder the future academic promotion and career advancement of women BBTM physicians; however, there was a slightly higher proportion of women in associate professor roles. Women had fewer total publications, but there was no statistical difference in publications after passing boards, suggesting that an emphasis should be placed on involving women in research during training. As most BBTM physicians go into academic careers, involving women in an equitable fashion when working with trainees to contribute to or lead investigations should be considered as a possible intervention moving forward. In spite of this, women BBTM physicians often hold important departmental leadership roles or have a prominent role in trainee education, which may contribute to earlier promotion relative to men in our study. Further longitudinal analysis need to be conducted to determine if leadership roles are contributing to earlier promotion among women.

Overall, these findings suggest that there is a need to address early gender disparities in academic medicine, as it takes time to foster

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TABLE 1 Early-career transfusion medicine physicians demographic and bibliometric information.

	Women	Men	
	Percentage [*N* or median (IQR)]	Percentage [*N* or median (IQR)]	*p* value
Gender	45% (58/128)	55% (70/128)	-
Academia	69% (40/58)	64% (45/70)	0.7
Rank			
Associate Professor	17.5% (7/40)	13.3% (6/45)	0.8**
Assistant Professor	82.5% (33/40)	84.4% (38/45)*	
Additional degrees	17% (10/58)	31% (22/70)	0.07
RCR	3.8 (0.7–12.4)	7.5 (3.1–25.7)	0.01
Number of publications per year since boards	0.7 (0–2.1)	1.3 (0.3–2.6)	0.2
Cumulative paper	6 (2–15)	9 (5–25)	0.03
Total citations	32.5 (6.8–195)	103 (16.5–279.8)	0.08
Citations per paper	7.7 (3.1–16.9)	8.5 (4.6–20.2)	0.33
First author	2 (0–5)	3 (1–7.3)	0.04
Middle author	4 (1–8)	6 (2–11.3)	0.06
Senior author	0 (0–2)	0 (0–1)	0.1

Abbreviations: IQR, interquartile range; RCR, relative citation ratio.
*One individual was still in training at the time of evaluation.
**A contingency table evaluation of assistant versus associate professor level was non-significant, and the *p* value for the evaluation was 0.8.

academic success for early-career physicians. Bibliometrics such as the number of publications, author line position and citations are predominantly used to determine the academic advancement of physicians by promotion and tenure committees. Such metrics compete with other responsibilities, such as patient care and education required from academic physicians by their departments. These metrics may impede the academic progression, career advancements and promotions of women physicians [5–7, 9, 11, 12, 17]. This slower rate of academic advancement may help explain the gender disparity in senior academic positions and the lack of women senior authors on manuscripts or editors of books [5–7, 9, 11, 12, 17, 19, 20]. Although there were more early-career promotions, it is unclear how these metrics affects progression to full professor or promotion to section or departmental leadership. In addition, the lack of author attribution to women BBTM physicians on manuscripts could be a reflection of women underrepresentation on the specialty journal editorial boards as editors and reviewers [21]. Having fewer women as editors contributes to fewer women being invited to write author commentaries and editorials in medical journals [22, 23]. Research shows that the lack of women leaders and mentors, gender stereotyping through differences in workplace expectations and disparity in career development opportunities impact the career advancement of women [19]. Addressing these imbalances requires a multifaceted approach encompassing institutional support, women mentorship and targeted interventions, such as increased NIH funding for women [24]. These solutions, along with addressing biases in the editorial evaluation process, move us towards creating a more equitable and inclusive academic environment [5].

There are several limitations to this study. We recognize the inability to fully account for the gender spectrum as a limitation of this study because of our binary use of the gender variable. Where appropriate, sex-based terminology utilized by the cited reference has been maintained. In addition, subgroup analyses may falsely attribute significance based on small numbers; however, given significant historical evidence showing disparities in academic support based on gender and the fact that this subgroup was selected a priori, our findings are not unanticipated [25].

In conclusion, our cross-sectional study sheds light on gender disparities in academic productivity, publication impact and authorship attribution among early-career BBTM physicians. By highlighting these disparities, this study contributes to the ongoing dialogue surrounding gender equity and prompts collective efforts to create a fair and supportive environment for all genders within the field of academic medicine.

ACKNOWLEDGEMENTS

T.S.I. designed the study, performed data collection and analysis and wrote and revised the manuscript; B.D.A. collected and analysed the data, performed data validation and edited the manuscript; G.S.B. designed the study and edited the manuscript; Y.C.T. performed data validation and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

T.S.I. is a consultant for Terumo Blood and Cell Technologies and Alexion Pharmaceuticals. G.S.B. received an education honorarium from Grifols Diagnostic, Inc.

DATA AVAILABILITY STATEMENT

Data will be available for review upon request by the publisher.

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ORIGINAL ARTICLE

Evidence that CD36 is expressed on red blood cells and constitutes a novel blood group system of clinical importance

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Abstract

Background and Objectives: Polymorphic molecules expressed on the surface of certain blood cells are traditionally categorized as blood groups and human platelet or neutrophil antigens. CD36 is widely considered a platelet antigen (Nak[a]) and anti-CD36 can cause foetal/neonatal alloimmune thrombocytopenia (FNAIT) in CD36-negative pregnant women. CD36 is used as a marker of differentiation in early erythroid culture. During the experimental culture of CD34+ cells from random blood donors, we observed that one individual lacked CD36. We sought to investigate this observation further and determine if CD36 fulfils the International Society of Blood Transfusion criteria for becoming a blood group.

Materials and Methods: Surface markers were monitored by flow cytometry on developing cells during the erythroid culture of CD34+ cells. Genetic and flow cytometric analyses on peripheral blood cells were performed. Proteomic datasets were analysed, and clinical case reports involving anti-CD36 and foetal anaemia were scrutinized.

Results: Sequencing of *CD36*-cDNA identified homozygosity for c.1133G>T/p.Gly378Val in the CD36-negative donor. The minor allele frequency of rs146027667:T is 0.1% globally and results in abolished CD36 expression. CD36 has been considered absent from mature red blood cells (RBCs); however, we detected CD36 expression on RBCs and reticulocytes from 20 blood donors. By mining reticulocyte and RBC datasets, we found evidence for CD36-derived peptides enriched in the membrane fractions. Finally, our literature review revealed severe cases of foetal anaemia attributed to anti-CD36.

Conclusions: Based on these findings, we conclude that CD36 fulfils the criteria for becoming a new blood group system and that anti-CD36 is implicated not only in FNAIT but also foetal anaemia.

Keywords

blood group antigens, CD36, erythropoiesis, Nak[a]

Highlights

- CD36 constitutes a new blood group system.
- CD36 is expressed at low level on reticulocytes and erythrocytes.
- Anti-CD36 can cause severe foetal anaemia due to its expression on erythroid progenitors.

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INTRODUCTION

The transmembrane glycoprotein CD36 (Figure 1) exhibits a broad distribution across various tissues. It is expressed in adipose tissue, mammary gland, epithelial cells, the membrane of placenta and many cancer cell lines [2]. Additionally, it is expressed in haematopoietic cells, including monocytes, platelets, and during the maturation of erythroid progenitors to red blood cells (RBCs) [3]. Individuals lacking CD36 may develop antibodies against the CD36 protein. The first case of CD36-related immunisation was reported from Japan, in a thrombocytopenic woman who became refractory to platelet transfusions [4]. Her antibody, anti-Nak[a], defined a platelet antigen with a prevalence of 97% among Japanese. Several family studies showed that Nak[a] antigen was inherited as an autosomal codominant trait [4] and Nak[a] was later shown to be carried on CD36 (Glycoprotein IV) [5]. CD36 deficiency is divided into two groups: type I deficiency, which is characterized by lack of CD36 expression on platelets and all other cells [6, 7] and type II, in which CD36 expression is lacking from platelets only [8, 9]. CD36 deficiency type I, while rare in Europeans, occurs with a prevalence of approximately 3% in Africans [10], 0.5%–1% among the Japanese [11, 12] and 0.5% in China [13], as recently summarized by Xu et al. [14]. Type I deficiency is of clinical significance since it can be considered a null phenotype associated with the development of antibodies against CD36. These antibodies have been implicated in various clinically important conditions, including platelet transfusion refractoriness [4, 15, 16], post-transfusion purpura (PTP) [17], foetal-neonatal alloimmune thrombocytopenia (FNAIT) [18], and transfusion-related acute lung injury (TRALI) [19]. Testing haematopoietic progenitor cell donors for CD36 type I has also been proposed due to the risk of incompatible transplantation [20].

Early work investigated the biochemistry, distribution and immunological characteristics of CD36 on different haematopoietic lineages in the adult and foetal settings [21, 22]. In line with this, CD36 now serves as a key cell surface marker in studies of human erythropoiesis using haematopoietic stem and progenitor cells (HSPCs). It is typically used in combination with CD34 to define the two earliest forms of erythroid progenitors, burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) [23]. During the culture of HSPCs obtained from adult peripheral blood or other sources, CD36 expression increases progressively and reaches its highest level in early erythroblasts at the end of the erythroid expansion phase in vitro. Subsequently, CD36 decreases during the terminal stages of erythroid differentiation [24].

During erythropoiesis culture experiments performed in our laboratory using peripheral HSPCs from random blood donors, we observed that one donor did not express the CD36 marker as otherwise expected, making it challenging to characterize the early stages of erythroid development. We therefore wanted to investigate the genetic basis behind the absence of CD36, which lasted throughout the culture but did not appear to affect erythroblast maturation compared with other donors positive for CD36. Since anti-CD36 is known to cause platelet-related clinical effects, we asked the question if the presence of CD36 on various stages of erythroid cells may be targets for these antibodies in a similar way known for blood groups expressed on early during erythropoiesis. Foetal anaemia due to suppression of erythroid progenitors has been found to be caused by immunisation against the KEL, GE, MNS and JR blood groups [25]. Indeed, reports implicating anti-CD36 in cases of hydrops fetalis and severe foetal anaemia have been described [26, 27]. Taken together, CD36 appears to have certain properties resembling a blood group molecule but the controversy regarding its presence or not on mature RBCs challenges the current dogma of how a blood group antigen is defined. Based on in vitro erythroid-culturing results from a blood donor that turned out to lack CD36, we performed additional experiments and scrutinized available datasets to evaluate if CD36 formally fulfils International Society of Blood Transfusion (ISBT) requirements to become a blood group system.

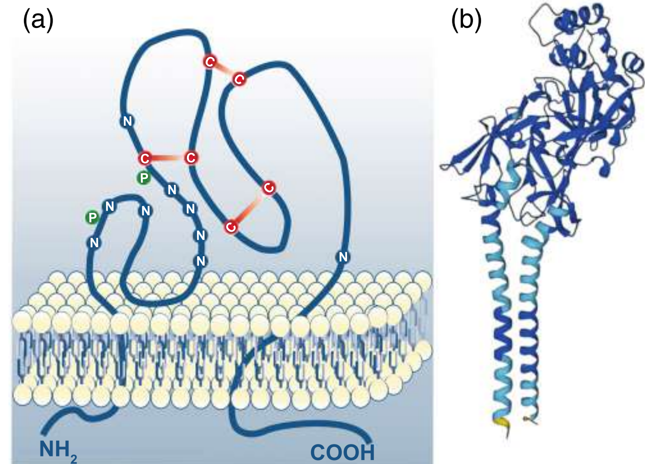

FIGURE 1 Structure of CD36 glycoprotein. (a) Schematic presentation of CD36 highlighting the transmembrane domains, nine confirmed N-linked glycosylation sites (N) (positions 79,102, 134, 205, 220, 231, 235, 247 and 417), two phosphorylation sites (P) (positions 92 and 237) and disulphide bonds between the extracellular cysteines (C) (positions 243–311, 272–333 and 313–323). (b) AlphaFold model of the structure of CD36 [1].

MATERIALS AND METHODS

In silico analysis of CD36 expression

HSPC and other haematopoietic cell microarray data were obtained from the Bloodspot website (https://servers.binf.ku.dk/bloodspot) [3] using the normal human haematopoiesis (DMAP) dataset [28]. RNA and protein expression data in erythroid cells from cultured HSPCs were downloaded from the supplement of Gautier et al. [24]. A search for peptides derived from the seven glycoproteins that bear the 35 reported human platelets antigens (HPA) listed in the HPA gene database (https://www.versiti.org/products-services/human-platelet-antigen-hpa-database/hpa-gene-database) was performed on the proteomics data from reticulocytes and RBCs, both membrane and soluble fractions [29, 30].

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Blood samples and ethics

RBCs, peripheral blood mononuclear cells (PBMCs) and platelets from donated whole blood units prepared in the Reveos automated blood processing system were obtained from anonymized leucocyte waste bags. Citrate-anticoagulated blood samples ($n = 20$) obtained as part of the routine blood donation procedure were anonymized and provided by the Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Region Skåne, Sweden, following approval (ref. no. 2022:19) in accordance with the Swedish research law.

Isolation and culture of CD34-positive HSPCs

PBMCs were obtained following Lymphoprep (Fresenius Kabi) gradient separation and further enriched for CD34 expression using magnetic beads (Miltenyi Biotec) according to the manufacturer's protocol. Equal numbers of CD34+ cells (starting with triplicates of 5000 cells/well in flat-bottomed 96-well plates) (Sarstedt) from each donor were cultured in a three-phase erythroid culture system and maintained at a concentration of $1–2 \times 10^6$ cells/mL throughout the culture as described previously [31].

Flow cytometry

Erythroid progression of differentiating HSPCs was analysed by flow cytometry at different days of culture as indicated, after labelling with a combination of the following antibodies: anti-CD34 (clone 581, BioLegend), anti-CD71 (clone L01.1, BD Biosciences), anti-CD235a (glycophorin A [GPA]; clone HIR2 (GA-R2), Thermo Fisher Scientific), anti-CD49d (clone 9F10, BioLegend), anti-CD233 (Band 3; clone BRIC6, IBGRL Research Products, Bristol UK) and anti-CD36 (clone 5-271, raised against human platelets as immunogen, BioLegend) with the corresponding IgG2a, κ isotype control (clone MOPC-173, BioLegend). Debris and dead cells were omitted by forward scatter and side scatter in combination with DAPI or 7-AAD staining (1:100 dilution before analysis). For peripheral blood analysis of reticulocytes, RBCs and platelets, the following combination was used: anti-CD235a, anti-CD61, Thiazole Orange (Retic-Count™, BD Biosciences) and either anti-CD36 or the same isotype control as above. Platelets from donors 1 and 2 were diluted 1:100 in PBS and counted in a haematology analyser (Sysmex kH3; Sysmex). Equal number of platelets from each sample was centrifuged at 800*g* and washed twice with PBS. Each sample was stained with monoclonal anti-human CD36 (BioLegend, clone 5-271) and isotype control (cloneMOPC-173, BioLegend). Platelets were washed with PBS and analysed by flow cytometry using the BD FACSCanto II (platelet analysis of donors 1 and 2), BD LSRFortessa (erythroid culture) or BD LSRFortessa X-20 (the blood samples from 20 random donors) instruments (BD Biosciences). FACSDiva software (v8.0-9.0, BD) was used for acquisition and FlowJo (FlowJo, LLC) for analysis of all the data.

RNA isolation and molecular analysis

Total RNA was extracted from cultured erythroblasts using the Mini-RNeasy Kit (Qiagen) following the manufacturer's protocol. Genomic DNA was removed using DNase I on-column digestion (Qiagen), then complementary DNA from the isolated RNAs was synthesized using Superscript IV VILO Master Mix (Thermo Fisher Scientific). CD36 primers encompassing exons 2-14 (CD36_cDNA22_43: 5′CCTGC AGAATACCATTTGATCC3′; CD36_cDNA1771_1752: 5′TTGGCCA CCCAGAAACCAAT3′) were designed using Primer BLAST based on the reference NCBI transcript NM_001001548.3 and synthesized by Thermo Fisher Scientific. PCR was performed using Phire Hot Star II DNA polymerase (Thermo Fisher Scientific) in a 20 μL reaction volume as follows: 98°C for 10 s, 35 cycles of 98°C for 10 s, 62.2°C 30 s, 72°C 90 s, then 72°C for 60 s, then hold at 4°C. PCR products were gel-purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific). Sanger sequencing was performed by Eurofins Genomics (Gottfried-Hagen) with the PCR primers. Sequence analysis was made with CodonCode Aligner v1.2.6 (CodonCode).

Statistical analysis

Statistical significance was calculated using Student *t*-test (two-tailed) with GraphPad Prism software. Statistical significance is indicated by asterisks: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$ in the figures. Error bars indicate the standard error of the mean (SEM).

Graphics software

Figures were partly generated in Servier Medical Art (Servier, France), licenced under a Creative Commons Attribution v3.0 unported licence (https://creativecommons.org/licenses/by/3.0). Colours were modified using Affinity Designer v2.

RESULTS

Erythroid culture reveals a CD36-deficient blood donor

During routine culture of HSPCs towards erythroid differentiation (Figure 2a) from random, anonymized blood donors, we observed a donor that appeared to exhibit a complete lack of CD36 expression at all stages of erythropoiesis. This finding was confirmed by parallel culture of HSPCs from a CD36-expressing donor with the CD36-deficient donor, here designated donors 1 and 2, respectively (Figure 2a). This finding presented a challenging situation as it made it difficult for us to separate the erythroid progenitor cells BFU-Es and CFU-Es based on their cell surface antigen expression [23] (Figure 2a-day 7). However, donor 2 cells showed an otherwise unremarkable antigen profile as maturation progressed (Figure 2a-days 14–21).

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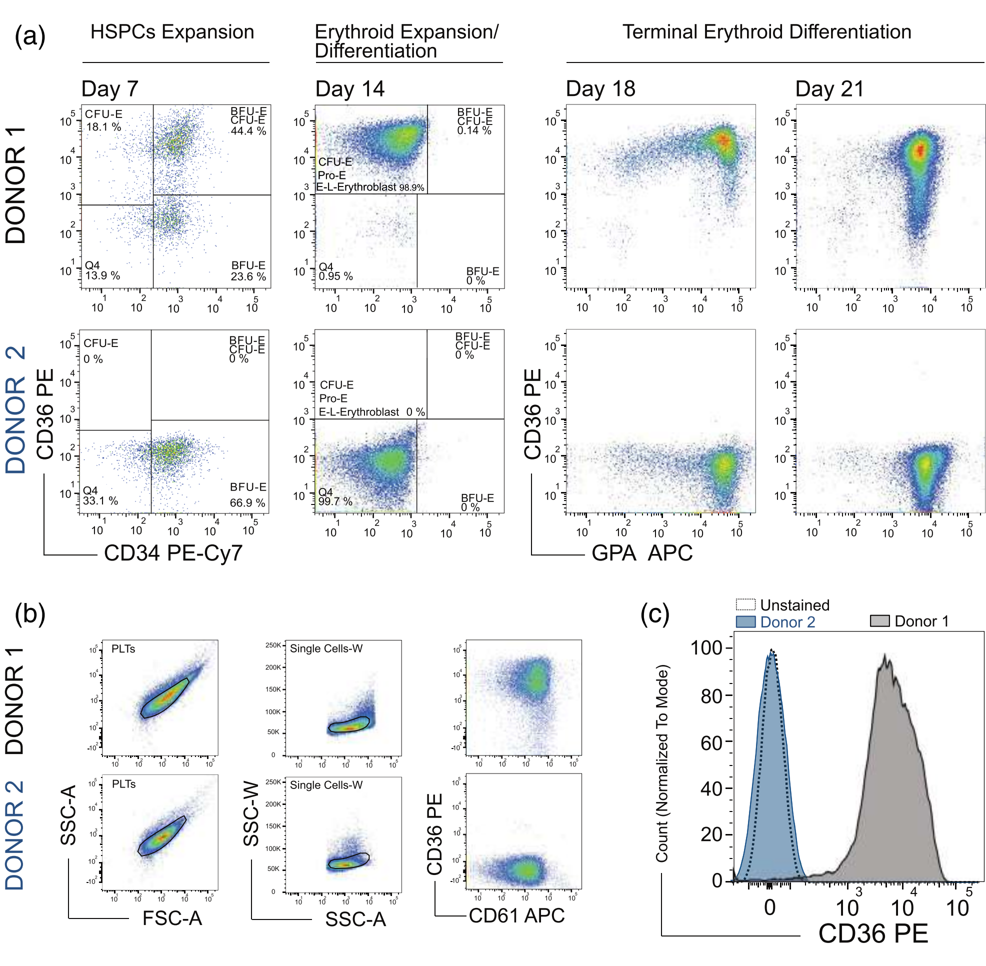

FIGURE 2 Lack of CD36 expression on HSPCs, erythroid cells and platelets. (a) Flow cytometry pseudocolor analysis plots showing CD36-positive cells (donor 1, top panel) and CD36-negative cells (donor 2, bottom panel) during erythroid culture (days 7–21). (BFU-E), (CFU-E), Pro-Erythroblast (Pro-E) and Early-Late-Erythroblast (E-L-Erythroblast). The timeline at the top represents the three stages of the erythropoiesis culture system used in this study. (b) Flow cytometry pseudocolor analysis plots showing the gating strategy and lack of CD36 expression on platelets from donor 2 (bottom panel) compared to those of donor 1 (top panel). (c) A histogram of platelets from donors 1 and 2 stained with anti-CD36 shows the complete absence of CD36 on donor 2 cells, comparable to the unstained control, while donor 1 cells are clearly CD36-positive.

Moreover, the CD36-negative cells throughout the erythroid culture (Figure 3a) underwent normal erythroid commitment by upregulating GPA and transferrin receptor (CD71) (Figure 3b-day 14). Donor 2 cells also underwent apparently normal terminal erythroid differentiation later in the culture, as characterized by the expected downregulation of CD49d and upregulation of band 3, the latter being a marker specifically associated with the maturing erythroblast (Figure 3b-days 18–21). These results suggest that lack of CD36 antigen expression does not affect human erythroid cell differentiation in vitro, and the absence of CD36 did not have a detectable impact on upregulating erythroid-specific cell surface markers during erythroblast maturation.

We also investigated the CD36 expression on platelets from both donors and showed that while the platelets from donor 1 expressed the expected, high levels of CD36, those of donor 2 lacked CD36 completely, consistent with the in vitro culture results (Figure 2b,c).

Genetic basis of CD36 deficiency in donor 2

DNA sequencing showed that donor 2 was homozygous for the SNV c.1133G>T (rs146027667) in exon 12 of the *CD36* gene on chromosome 7 (Figure 4a), encoding p.Gly378Val (Figure 4b). This SNV is predicted to have a deleterious effect based on scores from both Sorting Intolerant From Tolerant (SIFT) [32] and Polymorphism Phenotyping v2 (PolyPhen-2) algorithms [33] (Figure 4c). Analysis of gnomAD frequencies showed that this SNV is common in individuals originating from Middle Eastern countries and those of Latino/Admixed American origin (Table 1) [34]. The fact that CD36 is missing on both platelets and erythroblasts in donor 2 due to a germline variant previously implicated in CD36 deficiency in two other individuals [15], indicates that this variant indeed leads to CD36 deficiency but the latter study did not investigate CD36 on other cells than platelets. However, in line with the study by Toba et al. where all type I donors had CD36-negative erythroblasts [35], we conclude that the CD36

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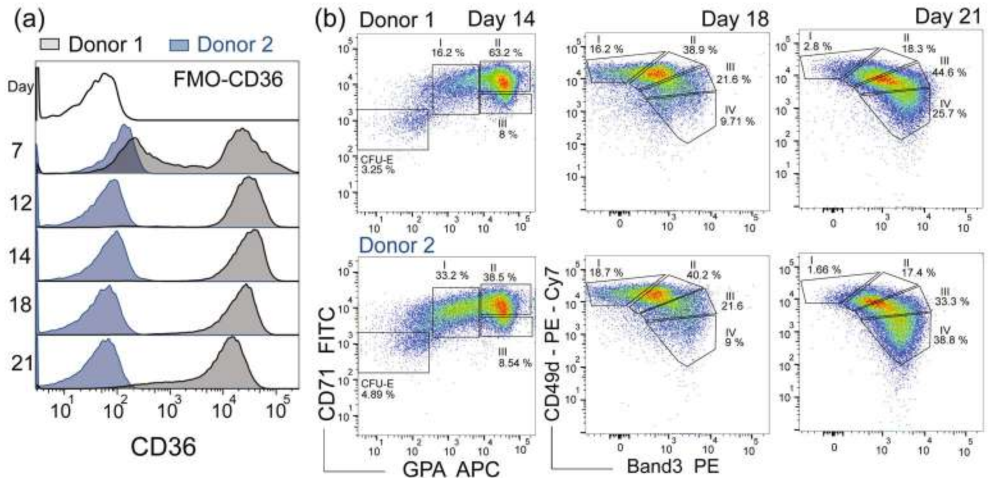
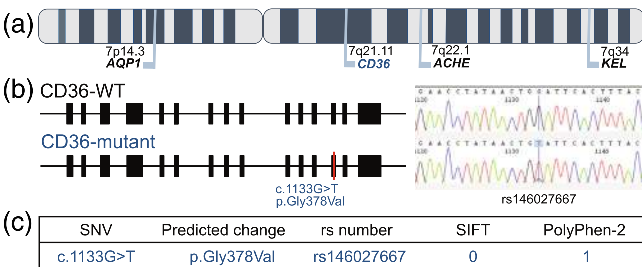


FIGURE 3 Lack of CD36 expression on HSPCs did not alter the erythroid lineage commitments nor the expression of erythroid-specific cell surface markers. (a) CD36 expression histogram overlay of donor 1 and donor 2 during the erythroid culture. (b) Flow cytometry pseudocolor plots showing CD36-negative donor cells (bottom panel) are differentiating towards erythroid cells like CD36-positive donor cells (top panel). CFU-E, (I) pro-erythroblast, (II) early-erythroblast, (III) late-erythroblast and (IV) orthochromatic-erythroblast.



SNV	Predicted change	rs number	SIFT	PolyPhen-2
c.1133G>T	p.Gly378Val	rs146027667	0	1

FIGURE 4 Genetic analysis of *CD36* in donor 2. (a) Chromosomal location of *CD36*: the gene is distant from other known blood group genes on chromosome 7. (b) Sanger sequencing of *CD36* transcripts from donor 2 revealed homozygosity for the SNV c.1133G>T in exon 12. (c) This SNV is predicted to have a deleterious effect based on SIFT and Polyphen-2 scores.

deficiency in donor 2 is most likely of type I, despite the absence of monocyte data.

CD36 has broad tissue distribution including erythroid cells

Analysis of publicly available mRNA and protein datasets showed that CD36 expression has a diverse tissue distribution (www.proteomicsdb.org) [36] and almost all haematopoietic cells express CD36 at different levels. Notably, it is significantly higher in the myeloid, megakaryocyte and erythroid lineages (Figure S1A). When differentiating HSPCs towards the erythroid lineage, the expression of CD36 is upregulated during culture and reaches its highest level around day 14 and then is downregulated during terminal erythroid differentiation (Figure 3a). In line with these data from our erythroid culture, similar expression patterns are observed for *CD36*-mRNA and CD36 protein at different stages of differentiation in other erythroid culture systems [24] (Figure S1B,C).

CD36 is expressed on reticulocytes and erythrocytes in peripheral blood

Currently, there is a controversy based on the limited data available regarding whether CD36 is expressed on the RBC surface or not. To further investigate the expression of CD36 on erythroid cells in peripheral blood, we conducted a study using 20 peripheral blood samples from random blood donors. To avoid a potentially false positive result due to platelets contaminating the evaluated erythroid cell population, we stained cells from peripheral blood with a combination of erythroid- and platelet-specific cell surface markers, GPA and CD61, respectively, then gated the cells as shown in Figure 5a. Interestingly, we identified and excluded from the analysis gate a subset of cells double-positive for these two markers (Figure 5a). Their size resembles that of normal RBCs and shares characteristics with both RBCs and platelets (Figure S2), indicating that they may represent platelets adhering to RBCs. Gating on the GPA + CD61−cells and adding a dye for RNA content further enabled the separation of reticulocytes from mature RBCs (Figure 5a). By using this gating strategy, we demonstrated that CD36 is highly expressed on platelets as expected (Figure 5b,c). Moreover, we could show a distinct but significant right shift both on reticulocytes and RBCs, indicating that CD36 is indeed expressed on these cells, albeit at low levels (Figure 5b,c).

In line with this, mass spectrometric approaches have been used to assess the overall proteome of highly purified reticulocytes and mature RBCs [29, 30]. We analysed these two datasets for the presence of CD36-derived peptides. One study identified six unique peptides specific for CD36 and found approximately five times higher levels of CD36-derived peptides in reticulocytes compared with RBCs.

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TABLE 1 Population frequencies of the *CD36* missense variant (rs146027667) identified in this study, in falling order of allele frequency, as reported in the Genome Aggregation Database (gnomAD v3.1.2, https://gnomad.broadinstitute.org/ [34]).

Population	Allele count	Allele number	Number of homozygotes	Allele frequency
Middle Eastern	3	316	0	0.009494
Latino/Admixed American	43	15,240	1	0.002822
Other	5	2086	0	0.002397
European (non-Finnish)	40	67,784	0	0.0005901
African/African American	4	41,376	0	0.00009667
European (Finnish)	0	10,594	0	0
Amish	0	910	0	0
East Asian	0	5198	0	0
South Asian	0	4834	0	0
Ashkenazi Jewish	0	3460	0	0

Notably, CD36 peptides were only found in the membrane fractions but not in the soluble phase [29]. The other study also reported six unique peptides from CD36 in mature RBCs, the vast majority in the membrane fraction of white ghosts [30]. Importantly, we analysed these two datasets for the presence of peptides corresponding to the seven reported HPA-carrying proteins but were unable to detect any such peptides, suggesting the data are indeed erythroid-specific. In summary, our investigation clearly shows that CD36 is present in the reticulocyte and RBC membrane.

CD36 fulfils the ISBT criteria to become a blood group system

Based on experimental data from this study, as well as analysis of data from the literature, we asked the question if CD36 fulfils the formal ISBT requirements to form a novel blood group system. As shown in Table 2, we conclude that this is now the case.

DISCUSSION

With every challenge comes opportunity. We encountered a donor lacking CD36 expression on erythroid progenitor cells, which provided us with a unique opportunity to address a question that is both simple yet controversial in the field of transfusion medicine. Can CD36 be considered a blood group antigen?

Traditionally, blood group antigens are defined by polymorphisms found on RBC surface molecules. Some, such as RH and MNS, antigens are mainly erythroid-specific, while ABO and KEL show broader tissue distribution [40]. Not unexpectedly, some blood group systems are shared between blood lineages. The Choline Transporter-Like protein 2 (CTL2; SLC44A2) is such an example that both underlies a blood group system on RBCs [41] and carries the HNA-3 antigens on neutrophils [42, 43]. Furthermore, several blood-group-carrying glycoproteins are expressed early during erythropoiesis [44, 45]. For example, antibodies targeting blood group antigens in the KEL and GE systems can cause antibody-mediated anaemia in the foetus and may even result in fatal hydrops [25]. In such cases, antibodies target the erythroid progenitors, which leads to suppression of foetal erythropoiesis and decreased RBC production. Since onset of CD36 expression occurs at the CFU-E stage, a similar mechanism is at play in pregnancies complicated by maternal anti-CD36, which could lead to suppressed erythropoiesis [26]. Reviewing the literature on anti-CD36, we found that it not only causes platelet-related disease including FNAIT but was also reported as implicated in several cases of foetal hydrops in pregnant women lacking CD36 [26, 27].

We show in Table 2 that CD36 fulfils the current criteria for a new blood group system according to the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology (WP-RCIBGT). As discussed above, antibodies have long been known to occur in $CD36_{null}$ individuals. Whether these antibodies are defined as alloantibodies or isoantibodies is less relevant to the WP-RCIBGT because existing blood group systems display examples of both. In analogy with CD36, antibodies against the A, B and RhD antigens are produced in the absence of the corresponding antigen carrier molecules, which has not prevented them from becoming the archetypes among blood groups. Additionally, the genetics underlying the absence of CD36 are already well-defined, including the proven independence from the other three blood group loci currently residing on chromosome 7. Thus, *CD36* meets all the ISBT criteria regarding inheritance and being an independent locus (Figure 4a and Table 2).

The categorisation of donor 2 as likely CD36-deficient type I relies here on the absence of CD36 from both platelets and cells of various stages of erythroid differentiation. Traditionally, lack of CD36 on monocytes has been used to define type I, partially due to their easy access in peripheral blood. Since monocytes were not available to us once we realized donor 2 lacked CD36, this can be considered a weakness of our study. It should, however, be noted that the classification of CD36 as a new blood group system does not depend on the definition of the deficiency type status of this donor.

Interestingly, the ISBT criteria have assumed but do not formally require a blood group antigen to be present on RBCs. This becomes particularly challenging in a case like CD36 where earlier erythroid cell stages express the glycoprotein at high levels while it has been difficult to detect on RBCs and reticulocytes in peripheral blood. In this study, we demonstrate that CD36 is expressed at low but significant levels on both reticulocytes and RBCs (Figure 5). The WP-RCIBGT may have to revisit its criteria regarding how much and where blood group antigens need to be expressed. Nevertheless, the WP-RCIBGT approved our proposal to make CD36 a new blood group system on 17 June 2023, substantiated not only by our experimental work [46] but also by a case report by Canals et al. in which anti-CD36 was reported to cause a weak panagglutination in routine RBC antibody testing.

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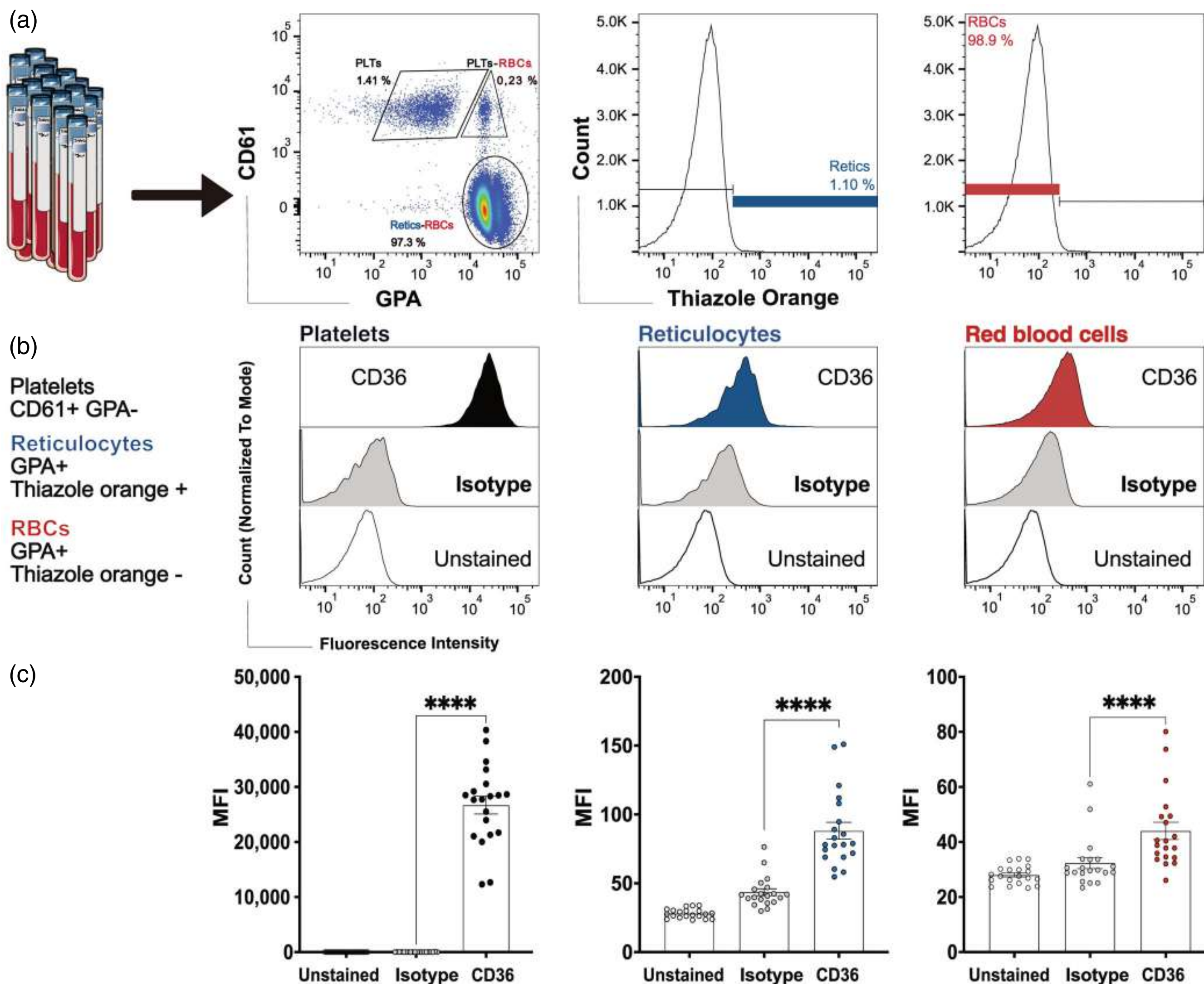

FIGURE 5 CD36 expression on reticulocytes and RBCs. (a) Pseudocolor plot showing the gating strategy used to identify platelets, reticulocytes and RBCs from peripheral blood samples using a combination of anti-GPA, anti-CD61 and thiazole orange. GPA + CD61− gated cells were further fractionated using thiazole orange, positive (reticulocytes) versus negative (RBCs). (b) Representative histograms displaying CD36 expression on platelets, reticulocytes and RBCs. (c) Bar graphs showing CD36 expression as mean fluorescence intensity (MFI) for the different cell populations. Unstained and isotype-stained cells were included as negative controls. Unpaired *t*-test analysis was used for statistical analysis (**** denotes $p < 0.0001$).

The antibody was identified in a woman of African origin whose newborn was diagnosed with NAIT [37]. More recently, Peyrard et al. also reported anti-CD36 in the plasma of more than 100 patients during routine antibody identification and showed that the antibody could be neutralized with soluble recombinant CD36 [38] giving unequivocal support for CD36 expression on normal RBCs. It is noteworthy that such reactivity has not been reported previously, particularly from Asian countries where most cases of anti-CD36 have been identified. It appears to stand in stark contrast to the serious erythroid consequences reported by Japanese and Chinese groups [26, 27]. We can only speculate about the reasons behind this apparent discrepancy, but it is possible that differences in immunohematological methodology may constitute an explanation, as well as underreporting of foetal anaemia, especially in the presence of thrombocytopenia due to anti-CD36. Interestingly, Kieffer et al. noted a size difference between platelet and erythroid CD36, which they suggested could be cell-lineage-specific and possibly an effect of differential glycosylation [22]. However, this has not been investigated further. No matter what, this and other reports will bring CD36 into the focus required for further study of these interesting questions.

In conclusion, we encountered a CD36-negative blood donor that prompted us to conduct a study including in vitro experiments, literature review and mining of publicly available proteomics data

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TABLE 2 Criteria for the establishment of new blood group systems.

ISBT criteria[a]	Yes/no	Evidence
The antigen must be defined by a human alloantibody.[b]	Yes	Multiple examples known incl. antibodies made by pregnant women [37–39] and causing foetal anaemia [26, 27]
The antigen must be an inherited character.	Yes	CD36 well known to be inherited [26, 27]
The gene encoding it must have been identified and sequenced.	Yes	Multiple variants known to cause the $CD36_{null}$ phenotype (Figure 4a) [39]
Its chromosomal location must be known.	Yes	CD36 is located at 7q21.11 (Figure 4a)
The gene must be different from, and not a closely-linked homologue of all other genes encoding antigens of existing blood group systems.	Yes	CD36 lacks significant homology with other blood group molecules. The three other blood group genes on chromosome 7 are distant and lack homology (Figure 4a).
Potential future ISBT criterion[c]		
The antigen should be expressed on erythroid cells normally present in the peripheral circulation	Yes	CD36 is expressed on maturing reticulocytes and red blood cells (Figure 5a–c) [29, 30]

[a]According to the ISBT Working Party of Red Cell Immunogenetics and Blood Group Terminology (WP-RCIBGT) (https://www.isbtweb.org/isbt-working-parties/rcibgt/blood-group-terminology.html).
[b]The ISBT WP-RCIBGT defines this as antibodies against an antigen the individual lacks.
[c]This additional criterion has been discussed by the Working Party on 17 June 2023, at the ISBT congress in Gothenburg and a revision will be formulated for approval at an upcoming Working Party meeting, along with revision of the antibody criterion discussed in the footnote above.

that resulted in acknowledgement of CD36 as a new blood group system.

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A.G.A., J.R.S. and M.L.O. conceived the study; A.G.A. and J.R.S. performed experiments; A.G.A. drafted the manuscript and created the figures. All authors analysed and interpreted the data and revised the manuscript. We would like to acknowledge the staff at the FACS Core Facility at the Lund Stem Cell Center for technical assistance.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available in the main paper and supplementary materials of this article. Additional data can be obtained from the authors on request.

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SUPPORTING INFORMATION

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

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REPORT

The future of blood services amid a tight balance between the supply and demand of blood products: Perspectives from the ISBT Young Professional Council

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Abstract

Background and Objectives: Blood services manage the increasingly tight balance between the supply and demand of blood products, and their role in health research is expanding. This review explores the themes that may define the future of blood banking.

Materials and Methods: We reviewed the PubMed database for articles on emerging/new blood-derived products and the utilization of blood donors in health research.

Results: In high-income countries (HICs), blood services may consider offering these products: whole blood, cold-stored platelets, synthetic blood components, convalescent plasma, lyophilized plasma and cryopreserved/lyophilized platelets. Many low- and middle-income countries (LMICs) aim to establish a pool of volunteer, non-remunerated blood donors and wean themselves off family replacement donors; and many HICs are relaxing the deferral criteria targeting racial and sexual minorities. Blood services in HICs could achieve plasma self-sufficiency by building plasma-dedicated centres, in collaboration with the private sector. Lastly, blood services should expand their involvement in health research by establishing donor cohorts, conducting serosurveys, studying non-infectious diseases and participating in clinical trials.

Conclusion: This article provides a vision of the future for blood services. The introduction of some of these changes will be slower in LMICs, where addressing key operational challenges will likely be prioritized.

Highlights

- In the future, blood services may offer new products, improve access to blood donation, implement new technologies and establish research partnerships with local health authorities.
- The pace of introduction of these changes will be slower in low- and middle-income countries, where addressing key operational challenges will likely be prioritized over implementing more costly and recent technologies.

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- Blood services should expand their involvement in public health research by establishing donor cohorts, conducting serosurveys, studying non-infectious diseases and even participating in clinical trials.

INTRODUCTION

Blood services manage the increasingly tight balance between the supply and demand of blood products. In high-income countries (HICs), the demand for red blood cells (RBCs) has substantially declined over the last decade [1]. In low- and middle-income countries (LMICs), the demand for blood products largely exceeds supply, thus severely limiting access to transfusion [2].

Moreover, the global demand for plasma derivatives and the production of source plasma have increased by >300% between 2000 and 2017 [3]. Yet, most countries largely depend on commercial plasma-derived medicinal products (PDMPs), which are manufactured from imported source plasma donated by remunerated donors.

Lastly, blood services are also increasingly involved in health research—especially since the pandemic [4]. They have conducted serosurveys, established biobanks, developed and validated diagnostic tests and participated in serosurveillance efforts and clinical trials [4–6].

These trends will affect the activities of blood services in the years to come. In this review, we explore how blood services may offer new products, achieve plasma and PDMP self-sufficiency, improve access to blood donation, participate in research activities to diversify and preserve their core business, and transition towards a personalized approach called precision transfusion medicine (PTM).

EMERGING AND NEW PRODUCTS

Whole blood

In many LMICs, whole blood remains the predominant blood product, given the lack of resources for multicomponent therapy. In HICs, whole blood has disappeared from the civilian setting with the advent of multicomponent therapy [7].

However, there is renewed interest for this product. In high-risk trauma patients, whole blood supports blood volume, oxygen delivery and haemostasis, thus helping achieve balanced resuscitation [7]. Moreover, whole blood is more convenient than multicomponent therapy in the trauma setting [7], where timely administration is key [8]. In two meta-analyses of patients with trauma-induced hemorrhagic shock, the risk of 24-h mortality and in-hospital/30-day mortality was similar among recipients of whole blood and multicomponent therapy [9, 10].

Nonetheless, these advantages must be weighed against some logistical pitfalls. To begin, reintroducing whole blood in a system optimized for multicomponent therapy will be challenging. This challenge may be further compounded in countries that largely rely on whole-blood-derived platelets, as reintroducing whole blood would reduce platelet availability. Another logistical pitfall is the shorter shelf-life of whole blood compared with red cell concentrates (RCC; 14–21 vs. 35–42 days) [11]—although waste can be minimized by reprocessing unused whole blood into RCC after 10 days [12]. Nonetheless, at least some HICs will likely make whole blood available in certain settings (e.g., trauma).

Cold-stored platelets

More than 50 years ago, cold-stored platelets (CSPs) were found to exhibit poorer viability than room-temperature (RT)-stored platelets (RTSPs) after infusion [13]. At that time, platelets were generally administered to prevent bleeding in patients with cancer, and so viability and duration of circulation trumped other quality parameters [14]. This evidence underlies today's practice of storing platelets at RT [14].

However, CSPs may offer benefits over RTSPs. To begin, they are less prone to bacterial contamination than RTSPs and hence have a longer shelf-life [15], which minimizes waste and (possibly) the risk of sepsis [16]. CSPs also release fewer pro-inflammatory mediators than RTSPs and exhibit a better metabolic and haemostatic profile [17]. Moreover, CSPs are better suited than RTSPs for therapeutic (but not for prophylactic) purposes [14, 18], and therapeutic platelet transfusions now account for a more significant proportion of recipients than they did 50 years ago [14].

Despite these advantages, maintaining a dual inventory of CSPs and RTSPs might be costly and logistically challenging. This limitation may be addressed through a new approach: storing platelets at 20–24°C for 4 days and transferring them to 2–6°C for an additional 17 days [19]. These 'delayed CSPs' were found to exhibit largely similar parameters as standard CSPs [19]. Another study successfully used this approach to address anticipated shortages amid the COVID-19 pandemic [20]. Evidently, where feasible, blood services should explore the possibility of introducing CSPs.

Cryopreserved platelets

Platelets are primarily used to prevent bleeding and control active bleeding. However, the storage of RTSPs for 5–7 days is logistically challenging, as it requires monitoring shelf-life and expiration while maintaining a stable temperature under continuous agitation. Moreover, supply shortages are regularly encountered and can be detrimental in remote environments or when large product quantities are mobilized.

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Cryopreserved platelets (CPPs) address many of these limitations. CPPs may be preserved for several years—typically in dimethyl sulfoxide, although newer protocols might be superior [21]. Thus, they can be mobilized on demand, potentially in large quantities [22], which is particularly useful for massive transfusions [23]. The risk of bacterial growth is also reduced, as cryopreservation obviates the need for RT storage. Moreover, studies evaluating the safety and efficacy of CPPs have demonstrated their potential haemostatic benefits [24, 25], notably in actively bleeding patients with thrombocytopenia [25]. Many studies have shown that CPPs are at least non-inferior to liquid-stored platelets for major bleeding (Table S1), and many HICs already use CPPs routinely in various contexts or have initiated evaluation and development programs [26].

Stem-cell-derived synthetic blood components

The production of stem-cell-derived products may address many challenges in transfusion medicine. Such synthetic blood products may greatly reduce—if not eliminate—the risk of transfusion-transmitted infections (TTIs). Synthetic blood products would also alleviate the pressure of a rising demand for conventional components.

In particular, synthetic RBCs could become valuable treatments. Alloimmunized patients and those with rare blood groups may significantly benefit from synthetic RBCs, as this supply would not depend on donor availability. Although the cost of synthetic RBCs will likely be prohibitive, part of it will be offset by the fact that the need for screening and extensive testing of donors for specific and rare RBC phenotypes would be considerably reduced.

Synthetic RBCs may also help spur the development of new treatments. Indeed, their long lifetime in the circulation may make them effective drug carriers [27]. For example, Rubius therapeutics developed synthetic RBCs expressing phenylalanine hydroxylase to treat phenylketonuria, a rare recessive disorder (although the company discontinued its ongoing trial) [27].

In vitro platelets are also promising to reduce the risk of alloimmune platelet transfusion refractoriness. They can be generated by differentiating induced pluripotent stem cells (iPSCs) into immortalized megakaryocytes progenitor cells, which subsequently mature and produce platelets [28]. One patient has received such iPSC-derived platelets and did not experience any significant adverse events after receiving three doses [29].

Cord-blood-derived red blood cells

The criteria for assessing the suitability of cord blood (CB) units for transplantation have become more stringent (e.g., higher thresholds for total nucleated cells and CD34+ cells) [30]. Although these changes have improved transplant outcomes, they have also reduced the number of CB transplants, resulting in more discarded donations.

However, such a waste of biological material could be alleviated if CB donations were repurposed for neonatal transfusion, which is especially common in preterm neonates [31]. Recent advances suggest that CB-derived RBCs could be transfused to neonates, and that their therapeutic effect might even be superior to that of standard adult RBCs. CB-derived RBCs can be fractionated from whole CB, and their characteristics are comparable to those of standard RBCs from adults [32]. In preterm neonates, the transfusion of CB-derived RBCs may be preferred to standard adult RBCs to increase the levels of haemoglobin F [33], whose levels must be elevated at this developmental stage to prevent retinopathy of prematurity (ROP). The BORN study is under way to compare the efficacy of CB-derived RBCs and standard adult RBCs for the prevention of severe ROP in extremely low-gestational-age neonates [34].

Other products

Convalescent plasma (CP) confers passive immunity and can be life-saving until more effective treatments are developed for a new or emerging infection. The value of CP may be even greater in LMICs, where the cost of newer treatments can be prohibitive [35]. The SARS-CoV-2 pandemic has emphasized the importance of contingency plans to offer CP amid a major crisis [36]. In response to the SARS-CoV-2 pandemic, many blood services worldwide established a CP collection program that included protocols on how to recruit CP donors [37, 38]. Although CP proved relatively inefficacious for SARS-CoV-2 [6], blood services may build on these experiences for rapid CP collection.

Another product that may be considered is lyophilized plasma, which can expedite plasma infusion in patients with severe coagulopathy or major bleeding [39]. French lyophilized plasma (FLyP) is a universally compatible product that can be reconstituted in less than 6 min [40]. Relative to fresh frozen plasma, FLyP can increase fibrinogen levels, shorten the time to transfusion, and improve prothrombin time ratio and factor II and V levels [40]. However, in patients with trauma-related hemorrhagic shock, lyophilized plasma plus RCCs was found not superior to 0.9% sodium chloride with respect to the composite outcome of episode mortality or impaired lactate clearance [41]. More studies are needed on the effectiveness of lyophilized plasma.

The production of lyophilized platelets could also be explored to extend shelf-life and expedite product administration in field settings. Lyophilized platelets are, however, not well characterized, with only two Phase 1 dose-escalation studies conducted to date [42, 43]. Some studies have also explored the feasibility of producing lyophilized RBCs [44–48], but this product is still in its infancy.

EMERGING TECHNOLOGIES AND INNOVATIONS

Pathogen reduction technologies

Pathogen reduction technologies (PRTs) encompass processes that reduce the risk of transmission of certain blood-borne infections [49].

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Current PRTs inactivate pathogens through UV illumination, intercalating agents or photosensitizers, or both [49]. PRTs inactivate a much wider range of pathogens than those currently screened by blood services in HICs. Their appeal thus stems in part from their potential to safeguard the blood supply against new infections. Another benefit of PRTs is the inactivation of passenger leukocytes, which raises the possibility that PRTs may eventually replace irradiation to prevent transfusion-associated graft-versus-host disease (TA-GvHD) [50].

However, most blood services consider PRTs unsuitable as a standalone pathogen mitigation strategy, that is, without other nucleic acid and serologic tests. As a result, their cost can be prohibitive.

Nonetheless, the future applications of PRTs are promising. The pathogen reduction capacity of an amotasalen/ultraviolet A system (for plasma and platelets) and an amustaline/glutathione (S-303) system (for RBCs) was recently shown to exceed or be comparable to the highest infectivity titres of West Nile virus, *Babesia microti*, dengue virus, Zika virus and chikungunya virus [51]. PRTs might therefore safely replace donor deferral and reduce the rate of donation withdrawal for these pathogens [51]. Should more effective PRTs emerge, the prospect of eliminating all pathogen-related deferral criteria may therefore not be unrealistic.

Point-of-care screening

Iron deficiency (ID) can be caused by frequent blood donation [52] and must be addressed to prevent anaemia and donor deferral. Ferritin testing is an appealing solution to prevent ID among blood donors. However, current tests are labor intensive and time consuming, and hence cannot inform iron reserves before donation. Therefore, blood services in HICs could develop or implement a point-of-care (POC) ferritin test to further improve donor care (see section 'Access to blood donation').

In LMICs, where some pathogens are highly prevalent, POC tests could be implemented for the most common infections leading to donor deferral or product withdrawal. Therefore, POC testing may alleviate the burden associated with asymptomatic carriers and reduce time-to-treatment initiation for test-positive donors. For example, in China, where hepatitis B virus (HBV) infections account for >70% of permanent deferrals [53], rapid pre-donation testing for hepatitis B surface antigen has been gradually implemented since 2010 (in addition to alanine transaminase testing) [54, 55]. Such a test was found to effectively capture HBV-infected donations [54].

A data-driven approach to collecting blood

The availability of comprehensive data on donors, donations and recipients has improved our understanding of the factors that affect donors' and recipients' health. In the United States, the domestic programs of the REDS III (Recipient Epidemiology and Donor Evaluation Study) and REDS IV-Paediatric studies link donor-, donation- and recipient-related variables to understand how their interplay influences recipient outcomes [56, 57]. In Sweden and Denmark, the second iteration of the SCANDAT databases ('SCANDAT2') offered nearly 50 years of donor–recipient follow-up data [58]. For the Swedish portion, a third iteration ('SCANDAT3-S') additionally includes routine blood chemistry results, thus allowing for studies on transfusion effectiveness and thresholds [59]. Blood services in other HICs should strive to implement similar initiatives to identify areas of improvement in donor or recipient outcomes.

ACCESS TO BLOOD DONATION

Blood services have significantly improved donor care over the past decades. For example, measures have been implemented to mitigate the risk of vasovagal reactions, and haemoglobin thresholds and inter-donation intervals have been adjusted to reduce the risk of anaemia and ID. Such progress is necessary to ensure a high rate of donor return, which helps maintain adequate reserves and might even lower the risk of TTIs (as repeat donors have a lower risk than first-time donors).

Another goal has been to make blood donation more accessible to racial and gender minorities. The sections below describe how blood donation could be made more accessible.

Cross-country disparities in blood donation access

Access to blood donation varies across jurisdictions. Several LMICs heavily rely on family replacement donors (FRDs), who tend to be first-time donors and, as a result, exhibit a higher risk of TTIs [60, 61]. However, many are committed to establishing a pool of volunteer, non-remunerated blood donors (VNRDs) and to converting first-time donors (FRDs and VNRDs) to repeat donors and have had some success [62].

Gender and racial disparities in blood donation access

In response to pressures from advocacy groups in HICs, blood services and regulatory authorities have sought to improve the inclusiveness of blood donation—a morally and rationally desirable goal that must be weighed against potential risks. Until recently, most countries indefinitely deferred gay, bisexual, and other men who have sex with men (gbMSM) from giving blood. In many HICs, this ban was progressively relaxed to time-based deferrals, without any safety impact [63]. Subsequently, many HICs have moved to a more inclusive, individualized approach that does not rely on sexual orientation. However, other countries have maintained the gbMSM deferral, given their local epidemiology of human immunodeficiency virus (HIV) and other pathogens, their culture and stakeholder input.

Nonetheless, some groups still face barriers to blood donation. Despite recruitment efforts, racial minorities remain underrepresented among blood donors in HICs, at least in part due to eligibility

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criteria [64]. For minority recipients, this limits access to compatible blood and must therefore be addressed. Some donors (e.g., gbMSM) also remain deferred because of their sexual behaviours or orientations. In particular, transgender persons may feel uncomfortable answering questions regarding past behaviours or experiences when they had their sex assigned at birth.

Blood services may explore some avenues to remove these barriers. The enrollment rate of racial minorities might be improved by educational approaches and multifaceted, community-based interventions, although the studies evaluating these strategies lacked robustness [65]. The known motivators and barriers among racial minorities may be used to design new interventions. Of note, blood services may consult the European Blood Alliance's 'Action Plan' developed by the Missing Minorities group [66]. Lastly, in many countries, more members of sexual minorities could be enrolled if advances in PRTs or diagnostic tests enabled the relaxation of deferral criteria for sexually transmitted diseases. Further, transitioning to an individualized, gender-neutral policy may significantly improve the inclusiveness of blood donation to sexual minorities.

PLASMA SELF-SUFFICIENCY

Few countries produce enough plasma to meet domestic needs. As a result, almost 90% of the global plasma supply is produced by the few countries that allow compensated plasma donations. Moreover, plasma demand has grown substantially over the past years [3], while supply has fallen amid the COVID-19 pandemic, leading to shortages.

Therefore, many blood services must explore new means of enrolling plasma donors to meet their self-sufficiency targets (although not all blood services are responsible for the collection and distribution of plasma, e.g., China). Compensating plasma donors may be one way to achieve self-sufficiency; however, in some jurisdictions, this would necessitate regulatory changes. Blood services may also collaborate with private sector partners to collect plasma from compensated donors (e.g., Canadian Blood Services). Additional plasma donation facilities may also be built to increase the plasma supply from VNRDs—an approach that has had some success in Canada and the Netherlands. In Europe, the SUPPLY project has been launched to improve the resilience of the chain of plasma processing and plasma collection from VNRDs, to enable a stable and adequate supply of PDMPs. Lastly, some blood services may consider increasing the allowed frequency of plasma donation, which varies widely from one country to another [67].

INVOLVEMENT IN RESEARCH EFFORTS

Outside the scope of the SARS-CoV-2 pandemic

Many blood services have a long history of working on pathogens alongside public health authorities (outside the scope of COVID-19). A number of studies have been conducted among blood donors to understand the seroprevalence and the rate of active infection of several pathogens (Table S2). Notably, one study evaluated the risk of *B. microti* based on an active tick surveillance program and donor serological testing [68].

Some blood services have also established donor cohorts to spur research on various conditions. For example, the Danish Blood Donor Study (DBDS) aims to identify genetic and environmental determinants of health among blood donors and the broader population [69, 70]. DBDS donors have, for instance, been used to study the colonization of *Staphylococcus aureus* in healthy individuals [71] and to explore the association between obesity and infections [72]. A subset of the DBDS, termed the DBDS Genomic Cohort [70], has been used to identify loci associated with various conditions [73, 74]. Setting up similar cohorts elsewhere holds promise to better understand diseases and determinants of health in various populations.

The contribution of blood services to research extends beyond serosurveys and longitudinal donor cohorts. For example, Hughes et al. documented the prevalence of obesity, hypertension, hypercholesterolemia and anaemia in young, first-time U.S. blood donors [75]. The estimates were aligned with those in the general adolescent U.S. population [75], suggesting blood donors are a valid data source to study these conditions. The authors also suggested that the role of blood centres may be expanded to include the detection, counselling and referral of donors with abnormal biomarkers [75], citing U.S. blood centres that provide such services. Another example is INTERVAL, a randomized controlled trial that originally aimed to identify the optimal frequency of whole-blood donation [76]—and whose data and samples have also been used to study conditions unrelated to blood donation [77, 78]. Clearly, blood services in HICs should strive to reproduce similar initiatives to expand their service offering.

During the SARS-CoV-2 pandemic

In HICs, the COVID-19 pandemic has expanded the role of blood services in research. Blood services have partnered with public health authorities to conduct SARS-CoV-2 serosurveys [5], which have helped inform public health decisions throughout the pandemic. Furthermore, blood services have helped expand our knowledge of COVID-19 immunity through the development of new tools [79] and the conduct of fundamental research [80, 81] and clinical trials [6]. Lastly, many blood services have established or plan to establish a longitudinal cohort of blood donors or a biobank, mainly for SARS-CoV-2 and TTIs [4]. It is hoped that these experiences will set the stage for more research partnerships involving blood services.

Strengths of using blood donors in health research

Partnering with blood services offers a number of strengths in health research. To begin, blood donors enable a disease's natural history to be studied before the onset of symptoms, since blood donors represent a healthy segment of the population [69]. Indeed, blood donors

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should be healthy at the time of donation but may become ill as they age; blood samples collected longitudinally—before the appearance of symptoms—may prove instrumental to identify early disease markers, which may in turn improve clinical outcomes. Moreover, the upfront cost of research initiatives involving blood donors (e.g., setting up a cohort) is minimal, since blood services already have the personnel, infrastructure and donor base in place to collect and screen samples [69].

Limitations of using blood donors in health research

Nonetheless, researchers must bear in mind some caveats associated with blood donor cohorts. To begin, many groups are either underrepresented among blood donors or outright excluded from blood donation [82], thus potentially limiting the extrapolation of results to the general population.

Despite this limitation, blood donors are a valid data source to study at least some diseases. In Japan, for example, the age-specific prevalence of hepatitis C virus (HCV) was similar in first-time blood donors and adults ≥40 years who were screened for HCV [83]. Furthermore, the 'healthy volunteer' bias typically observed in blood donors (i.e., the fact that blood donors tend to represent a healthy segment of the population) may not be addressed by alternative data sources or study designs, such as setting up a cohort from the general population (e.g., UK Biobank) [84]. The representativeness of blood donors may be improved by removing some donor selection criteria or conducting blood drives in rural areas or areas with underrepresented racial groups. Various statistical adjustments (e.g., inverse probability weighting) may also address this limitation.

Another limitation is that most donations collected by blood services consist of whole blood, for which the minimum interval between donations is significantly longer than that for apheresis donations. As a result, longitudinal studies on RBCs—but not on other blood components—may be limited by longer intervals between donations.

PRECISION TRANSFUSION MEDICINE

PTM is an innovative approach that considers individual patient attributes to improve outcomes and safety. This novel approach has been enabled by advances in data analytics and bioinformatics. Specifically, PTM considers a patient's genetic factors (e.g., blood type compatibility, genetic markers), medical background (e.g., patient with chronic anemias, surgical patient) and immune status (e.g., alloimmunization) to select the most appropriate blood components and transfusion strategies.

In addition to enhancing transfusion efficacy, PTM helps understand the interplay between patient factors and transfusion outcomes. For example, the storage and transfusion of RBCs are currently guided by simplistic workflows (i.e., 'first-in-first-out' principle to avoid wastage); however, the recent studies that have used a PTM framework have shown that the quality of stored RBCs (as measured by haemolysis) is influenced by donor characteristics (including genetics) as well as temporal and process-specific factors [85]. This suggests that the post-transfusion functional integrity of RBCs and patient outcomes could be enhanced with careful donor selection and storage policies.

OUTLOOK FOR LOW-INCOME AND MIDDLE-INCOME COUNTRIES

Clearly, the path to improving access to (and the quality of) blood products will significantly differ among HICs and LMICs. The timeline for implementing the vision laid out in this article should therefore be adapted to a country's resources, healthcare system and culture. Many LMICs will prioritize other operational goals—such as establishing a national blood service to oversee the centralized blood collection system of VNRDs—before tackling the challenges encountered by HICs.

Rather than introducing new products, blood services in some LMICs may instead prioritize improving the quality of current products and implementing multicomponent therapy. This may reduce the rate of product waste, which remains high primarily because of TTIs [86].

The emerging technologies discussed herein will also be introduced later (and perhaps differently) in LMICs. For example, LMICs that do not routinely perform nucleic acid testing (NAT) may need to choose between NAT and PRT. According to a modelling study in Uganda, PRT combined with rapid diagnostic tests may be superior to serological testing (alone) to reduce the incidence of HIV, HBV, HCV and malaria [87]. Moreover, PRTs inactivate passenger leukocytes and may thus be particularly effective at reducing the incidence of TA-GvHD in LMICs [50], where non-leukoreduced whole-blood components are commonly transfused. Clearly, more cost-effectiveness analyses are needed to assess the costs and benefits of PRT in LMICs.

Similarly, in LMICs, achieving plasma self-sufficiency may not be a realistic short-to-medium-term goal. Limitations to improving plasma access include the prohibitive cost of plasma fractionation, imported PDMPs and plasmapheresis kits, and the suboptimal quality (e.g., presence of infectious disease markers) and processing (i.e., unmet recognized standards) of recovered plasma [88]. In some LMICs (e.g., Indonesia), minimizing the waste of imported PDMPs may be a more practical short-term goal [89].

Moreover, establishing donor cohorts may not be a high, short-term priority in LMICs. Many of these countries currently lack the molecular tests (e.g., NAT) to detect TTIs, and so devoting resources to other tests (unrelated to transfusion safety) would not be a sound strategy.

This article laid down a vision of the future for blood services. Specifically, blood services may offer new products (e.g., whole blood [in HICs], cold-stored platelets), improve access to blood donation (e.g., for gbMSM), implement new technologies (e.g., PRTs) and establish research partnerships with local health authorities. The pace of introduction of these changes will be slower in LMICs, where

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addressing key operational challenges (e.g., reducing reliance on family/replacement donors) will likely be prioritized over more costly and recent technologies. Blood services in LMICs may interpret our vision as a long-term complement to some of their more pressing objectives.

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The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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SUPPORTING INFORMATION

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

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OBITUARY

Dirk de Korte (1955–2024)

On March 22, 2024, Dirk de Korte, PhD, passed away at the age of 68. Dirk started his career in 1981, when he studied the purine and pyrimidine metabolism of normal and leukaemic blood cells at the Central Laboratory of the Blood Transfusion Service from the Netherlands Red Cross (CLB, Amsterdam, the Netherlands, currently Sanquin Blood Supply). After the defence of his thesis, in the early days of development of the buffy coat method to produce platelet concentrates, he started working on optimisation of blood component transfusion medicine together with Hans Loos. Dirk performed studies on overnight hold of whole blood, development of butane-1,4-diol cooling plates, and leukocyte removal, ultimately resulting in a production method that could easily be executed in day-to-day routine blood banking. He worked on formulation of the early platelet additive solutions, later commercialised as PAS-D. Also, Dirk invented PAG3M for storage of red cells, succeeding in the maintenance of crucial storage parameters ATP and 2,3-DPG. To examine their clinical effectiveness, he developed a biotin labelling method that is now routinely applied. Associated with this, Dirk worked on changing the plasticisers in blood bags in order to remove DEHP, which will be banned from being used in medical devices in Europe in 2030.

Dirk's most significant contribution to the field of transfusion medicine was the development of a 'diversion pouch'. The idea was based on the notion of Prof. Blajchman that the skin plug contains many bacteria. It was indeed shown by Dr. Olthuis and colleagues that the first millilitres of collected blood have a high level of bacteria. Dirk used this information to develop a bag system that contained a diversion pouch in the collection tubing so that the first 20 millilitres of whole blood, containing the skin plug, could be diverted. This reduced the infectious risk of the blood collected in the main blood bag. At the time, it was estimated that 1:3000 platelet units contained bacteria, leading to morbidity in 1:50,000 units transfused with an associated mortality rate of 1:500,000. Dirk showed that introduction of the diversion pouch more than halved the infection rate of platelet units. This practical solution to a clinically relevant problem quickly found acceptance in other blood centres. Considering that millions of platelet concentrates are transfused per year, many lives have been saved by this invention.

Dirk retired from his position as manager of the department of Product and Process Development of Sanquin Blood Bank in 2021. However, he remained engaged and was always available to answer questions.

He was internationally recognised as an expert on blood components and standardisation thereof. He was a member of various international advisory boards, chair of the Dutch blood products guideline committee and the Dutch representative for the European Union committee that sets the guidelines for blood products. Dirk gave many presentations all over the world and thoroughly enjoyed passing on his knowledge to younger generations. He published over 200 papers and assisted six PhD students.

In November 2023, Dirk received the message that he was terminally ill. His faith led him through this difficult time. *Rejoicing in hope, patient in tribulation, continuing steadfastly in prayer* (Romans 12:12), it said on his mourning card. He was a loving husband, a great father and a wonderful grandfather to his grandchildren. He will be missed by his peers as senior adviser and thoughtful, knowledgeable, but most of all, a warm human being.

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EVENTS

See also https://www.isbtweb.org/events.html	
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